PHYTOPATHOLOGY

VOULME XVI NUMBER 4

APRIL, 1926

BACTERIAL STALK ROT OF CORN[1]

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In 1919 the writer (14) called attention to a little known disease of corn prevalent in Arkansas. This was followed by two short papers in which brief descriptions of the disease and the pathogene concerned with it were presented. The present paper aims to present a thorough but succinct account of the disease and the pathogene.

DISTRIBUTION AND EXTENT OF DAMAGE

Subsequent to the first communication (14), the disease has been reported by other investigators from the following states: New York, North Carolina, Mississippi, Louisiana, Ohio, Illinois,[2] North Dakota, and Arizona.[3] It is possible that in some instances this disease may have been confused with some other malady, but it is quite probable that the disease is present in other states, as well as in most of those mentioned above. As far as Arkansas is concerned, and probably other southern states, the pathology of corn in general is hardly fathomed as yet.

As to the presence of this disease in other countries, there are no published records known to the writer with the exception of that by Dawson (6) of East Africa, whose report is so brief that there is some question as to the disease described by him being the same as that here discussed.

Evidence will be presented later which clearly indicates that this is a disease which does damage only during moist, muggy, warm weather, and that regions in which the air temperatures are much below 27° C. during the growing season are likely to be free from it. The disease is very probably present in all the states along the Mississippi River from Illinois southward.

[1] Published with the permission of the Dean of the College of Agriculture, and Director of the Arkansas Agricultural Experiment Station.

[2] U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Bul. Supplement 21. 232–233. 1922. (Mimeographed.)

[3] *Ibid.* Supplement 27. 238–239. 1923. (Mimeographed.)

The writer has noted this disease in Arkansas every year since 1919 (6 years). Field observations have been made and specimens received from a wide area of the State, including the Mississippi River (eastern), as well as the Ozark (western) section. During the years when summer rainfall is quite low, the disease does very little damage and does not cause losses of more than one to two per cent. But when the late June, July, or August precipitation is more than usual, as occurred in some sections of the State in 1920 and in 1925, the disease is by far the most destructive one of corn in the State. I have already recorded losses of from 10 to 30 per cent of the stalks in some fields (15). In 1924 the disease was noted in epidemic form at Fayetteville, Ark., in a small garden of sweet corn—11 out of 20 hills of Golden Bantam having the disease and other varieties being affected to a lesser extent. During 1925 quite a few reports and specimens were received from a number of counties in the eastern part of the State, including Randolph, Independence, and Jackson Counties. Some of the correspondents who reported considerable damage, and who also sent specimens for identification, wrote as follows: "I am sending you specimens of corn stalks that have become affected with what appears to be stalk rot. This corn appeared to be in a healthy condition until a few days ago. Now many stalks are falling over and are being attacked to a more or less extent." (Extract from letter of E. Y. Fitch, June 29, 1925.) "This is the only field I know of that is damaged, but there is considerable damage in it." (Extract from letter of J. E. Critz, June 27, 1925.) "I am sending you herewith specimens of stalks of corn that have us somewhat puzzled as to what is the cause of the rotting off just at the top of the ground. This condition is quite prevalent in this county (Independence County), especially in rich delta or bottom lands—and in some instances is materially affecting the stands. This corn has not suffered seriously from moisture, nor has the temperature exceeded about 98 to 100 degrees F., and at this time there is plenty of moisture, but not excessively wet." (Extract from letter of S. C. Knight, June 27, 1925.)

As to the damage done by this disease in other states, Edgerton's report[4] is of interest. "The bacterial stem rot of corn described by Rosen from Arkansas has been known in Louisiana since 1908. I saw it the first year that I was in Louisiana and have seen it to some extent nearly every year since. Some years it is very troublesome in some localities and other years it is of little importance." This corresponds closely to the writer's observations in Arkansas. From Illinois, Tehon (20, p. 197) reports as follows on the disease—"It was found in Illinois in 1921 in two counties, Jackson and Randolph, where it was reported as serious in the fields affected by it. In

[4] U. S. Dept. Agr. Bur. Plant Indus. Plant Disease Bul. Supplement 21. 232–233. 1922. (Mimeographed.)

1922 it was again found in small amounts in a single field in Union County, where it was reported to be causing the death of one per cent of the stalks. In 1923 it was seen in Alexander County, where an examination of 100 acres revealed two infected stalks." Roy C. Thomas, in a letter to the writer dated September 29, 1924, reports the disease on Golden Bantam and Country Gentleman in Ohio.

As already indicated, the disease is to be looked for primarily in the southern portion of the corn growing section, although it appears that it may develop to some extent farther north (probably during exceptionally moist, warm periods). In addition to the varieties previously mentioned (15) as susceptible to the disease, the following sweet corn varieties have been found susceptible: Golden Bantam, Stowell's Evergreen, and Country Gentleman.

DESCRIPTION OF THE DISEASE

The writer has already discussed the symptoms in former publications (14, 15). Additional information enables me to make some corrections and

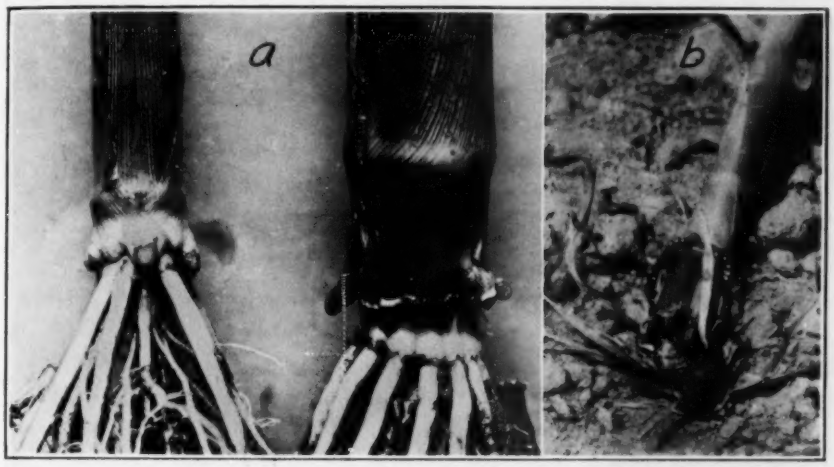

FIG. 1, a—Plant on right artificially infected at a lower node, the resulting rot involving several adventitious roots as well as stalk tissue. Plant on left served as check. b—Natural infection at a lower node which included root infection.

additions to these. The disease is primarily one affecting the stalks and leaves. The name "root-rot," that was first applied (14), is misleading. It was used because the first observations showed the roots as the main seat of disturbance, but further study has convinced the writer that this was due to some other cause which happened to accompany the bacterial disease in the field where it was first observed. The compact, matted appearance of the roots, as first described, has not been observed in any subsequent ma-

terial, and the writer is inclined to think that this was largely due to wire-worm injury, the matting of the roots representing successive efforts on the part of the host to replace the elongated roots cut short by the wire-worms. Also, too much emphasis was placed on the purplish-brown discoloration of the interior of lower nodes, this feature being more or less common to a number of root troubles (and perhaps non-parasitic root troubles), and is

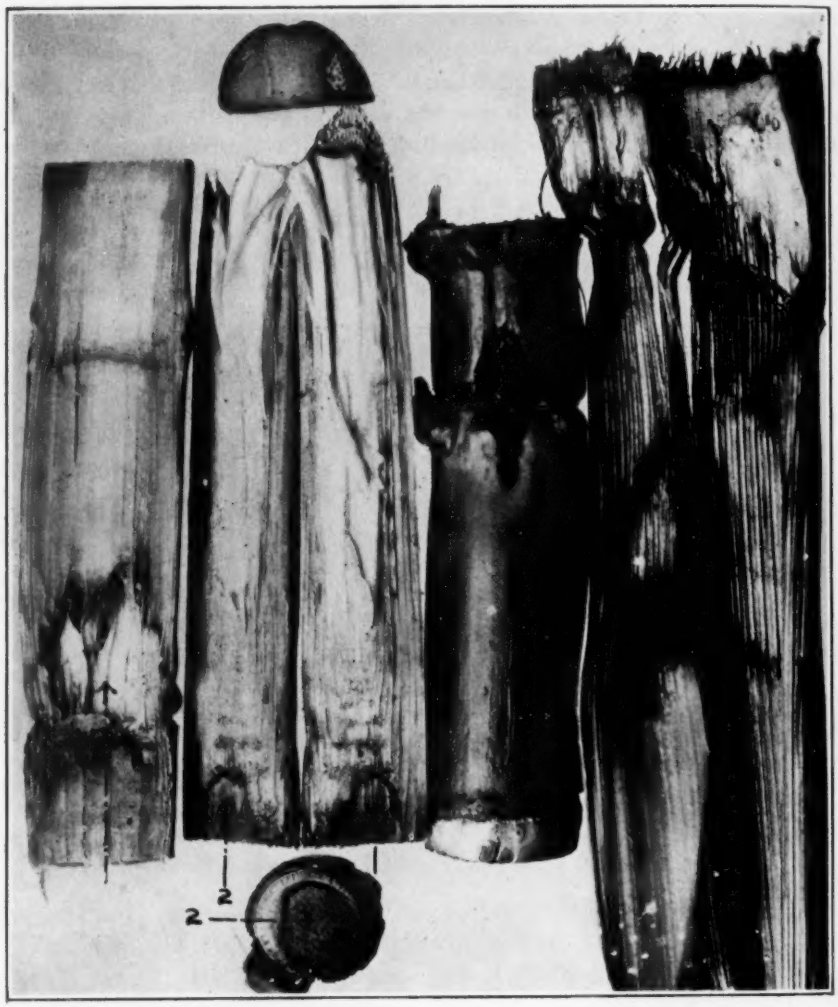

FIG. 2.—Natural infections; arrow at extreme left pointing to internal pocket of dry rot or later stage, other arrows (2) pointing to an early, wet rot stage; sheath at the extreme right showing discoloration and disruption of tissue occurring at an infected node.

only significant in this disease when accompanied by a characteristic rotting of the same or adjoining tissue (Fig. 1).

Briefly stated, the symptoms are as follows: (a) A light-brown or dark-brown rotting, and premature dying of the lowermost leaves (upper leaves rarely become infected), usually beginning in the parts joining the stem and confined primarily to parts of the sheath (Fig. 2); (b) a yellowing and withering of the leaf blades whose sheaths have become rotted; (c) a rotting of the stalk, usually at a lower node (Fig. 2), often at the node immediately above the soil line; the rotted part in severe infections is a dark-brown or blackish, soft, putrid, sunken mass, often with a grayish-white slime in recent infections, and light-brown, shriveled and broken up in older infections (Pl. XVII). In most cases the infection does not extend through the whole thickness of the stalk, but either confines itself to a small localized blackish spot with a water-soaked margin, extending not more than 0.25 inches into the interior and invading only a small part of the nodal circumference (Fig. 4), or the infection, beginning at some external point on the node, extends into the interior, invading a considerable part of this without showing much at the surface; or lastly, the rot may involve the whole circumference of the node and extend but a short way into the interior. As formerly reported (15), the complete rotting of a lower node and the subsequent falling over of the stalk are symptoms of this disease in severe infections (Fig. 3). Roots which happen to be attached to those parts of the stalk which are diseased will likewise become infected, but it should be noted that this disease is primarily a stalk and leaf rot and not a root rot; likewise that leaf blades in contrast to leaf sheaths are rarely infected, though the author has often succeeded in infecting these artificially (Fig. 5).

It is often difficult to distinguish this disease on the leaf sheaths from leaf spotting due to other causes, particularly those spots which are associated with the presence of pollen sacs and aphis honey dew in the ventral side of the sheath, and which, according to Durrell (8 and 9), make it possible for normally saprophytic fungi and bacteria to penetrate the sheath and produce discolored spots. However, such spotting, in the writer's observations, is found mostly on the leaf sheaths, while the bacterial disease here described is conspicuous as a stalk infection. Furthermore, the bacterial disease is found on young as well as on older plants (Fig. 3), while Durrell notes that the Diplodia dry rot fungus is found mostly on older plants.

What is the explanation for the prevalence of this disease on the lower parts of the plant? In other bacterial diseases which are not systemic and which are not particularly associated with insect dissemination, primary infections are also apt to appear on parts close to the soil line. Black rot

of cabbage, black leg of potato, and angular leaf spot of cotton immediately come to mind as good illustrations of this. The same thing is true for a large number of fungous diseases. Closeness or contiguity to soil is the main explanation, the pathogene either being associated with the seed or more directly with the soil. In the latter instance the pathogene lives over on formerly diseased material of past crops or, rather rarely, lives a more or less independent existence in the soil. In these instances dissemination is largely by driving rains or by surface drainage. The angular leaf spot of

FIG. 3.—Typical stalk rot close to the soil involving an extreme disintegration of localized stem tissue.

a—Rot of older plant.

b—Rot of young plants; plants prostrate as a result of extreme rotting. Photographed at Burdette, Ark., June 27, 1921.

cotton is a very good illustration of this. In addition to closeness of soil, the frequency of injuries around the crown of the plant, either in the form of natural wounds or in the form of insect and other injuries, also play an important rôle in rendering the lower parts of the corn plant more subject to disease.

ISOLATION AND ARTIFICIAL INOCULATION EXPERIMENTS

It has previously been reported that the pathogene is rather easily isolated (15). During the six years that have elapsed since its discovery, it has been isolated more than 50 times from natural and from artificial infec-

tions. Owing to the great abundance of bacteria in infected portions, a small bit of material from any portion of the rotted tissue is usually sufficient for microscopic examination. Under the pressure of a cover glass, grayish-white clouds of the bacteria ooze out of such tissue floating in a drop of water. Not infrequently other microorganisms, including various fungi and comparatively large-sized or motile bacteria, are also to be seen in rotted tissue, but if care is taken to choose only freshly infected tissue, these small, plump rods are quite frequently the only microorganisms to be observed. Within the parenchymatous tissue of the corn stalk, which it will be recalled occupies a considerable part of the stalk, the bacterial pathogene thrives and multiplies rapidly under suitable conditions of moisture and temperature, and infected cells are filled with bacteria. The writer has never observed the pathogene in the vascular tissues, and, as the rotted region is always more or less limited in extent, the disease may be considered as a localized one. It may be possible to have localized infections of vascular systems, but the fact that this disease often leaves only the shredded fibers or vascular elements at the rotted joints is external evidence of the lack of vascular infection (Pl. XVII, a). Conceivably, should any injury occur to the xylem of the amphivasal corn bundle so that the phloem would be exposed, it also might become infected.

Isolations by means of platings from internal tissues, either without subjecting this interior tissue to a germicide, or submitting it to the action of mercuric chloride for the customary minute or two and then washing in sterile water, are very likely to result in the development of one type of colony (Pl. XVIII). In order to be certain that any fungus, which might be present and which might be lost by bacteriological dilution culture methods, is isolated, bits of unmacerated tissue were surface sterilized, and plated out directly on various media, especially potato glucose agar and nutrient agar. This, in conjunction with the dilution plate method, should enable one to isolate fungi as well as bacteria which may be present. As a result of a large number of such trials, the writer was able to establish early in his work the presence of a certain bacterium in the diseased tissue. Of the fungi which have been encountered in such rotted areas, *Rhizopus* spp. and *Fusarium* spp. lead in frequency. But inasmuch as the tissue harboring these fungi was always teeming with bacteria, while many isolations revealed no fungi, there is no good reason for considering these other than as secondary invaders. Occasionally other species of bacteria have been isolated from diseased material, but having once learned to distinguish the pathogene, other bacterial colonies which appeared sporadically were readily explainable as secondary invaders or as contaminations.

Artificial inoculations have been made on somewhat more than 2,000 plants, involving about 150 separate trials in the field and greenhouse, and

typical symptoms have been obtained from approximately 20 per cent. It was discovered early in the work that infections were not always obtainable. These failures at times could not be ascribed to any lack of virulence on the part of the organism, as success and failure occasionally occurred in different trials made but a few days apart. As failures became at times more common, and typical infections discouragingly rare, it seemed necessary to make a thorough study of environmental factors as related to disease inception and development. Furthermore, sporadic outbreaks of the disease under field conditions, which were noted at times as regular epidemics in some localities, followed by a more or less rapid and complete subsidence of the disease, made it all the more desirable to undertake such a study.

Dr. Charles F. Hottes, of the University of Illinois, very kindly permitted the writer to use the excellent control apparatus which he had constructed. This consists of a series of large glass chambers in which temperature and humidity, and light to some extent, can be accurately controlled. Time permitted only a study of the effect of temperature on infection. Three different series were run in these chambers at different times, and the results check up closely in the three. As far as humidity is concerned, it had previously been ascertained that little or no infection was secured in a dry atmosphere during the incubation period, and that to insure infection a very moist or practically saturated atmosphere was necessary. This was obtained by wetting large strips of filter paper and placing them on the inside of large bell jars next to the walls. All plants, checks as well as those inoculated with bacteria, were treated the same way. Inoculations were made by spraying the plants with fresh cultures of the bacteria growing upon nutrient agar slants. The spraying apparatus consisted of sterilized, all-metallic artist's syringes. Wounded, as well as unwounded, plants were treated, the wounds consisting of needle pricks and knife incisions on stems, usually at the base.

Summarizing the results of these experiments, it was found that below 20° C. there were no infections; between 20° and 25° C. infections were few and much limited in the size of tissue affected and in the severity of infections; between 25° and 30° C. infections were more numerous, the size of infections greater, and the discolorations and disintegrations of the affected cells much more severe; between 30° and 35° C. the greatest number of infections was obtained, the affected area showing the greatest amount of disintegration and death of tissues.

A portion of the notes of one of these series reads as follows: December 1, 1922, 11:00 A. M., sprayed one pot of corn (Neil's Paymaster) with Burdette strain, another pot with Jackson County (Illinois) strain, and one with sterile water. Three such pots placed in each chamber at the following temperatures: 15°, 20°, 25°, 30°, and 35° C. Bell jars lined internally with wet filter paper placed over each pot.

December 2, 9:00 A. M.—35° chamber reads 36° C. Others O. K.

December 3, 8:30 A. M.—35° chamber reads 40° C. Others O. K. Bell jars removed. In 30° chamber, yellow tips noted on several leaf blades of pots of both strains, one leaf showing withered area around margin.

December 4, 8:30 A. M.—Plants in 35° chamber showing brownish-yellow tips in pots of both strains, no leaf discoloration of plants sprayed with sterile water. Same observations on the plants in the 30° chamber. In the 25° chamber, two leaves with yellow tips in the pot sprayed with Burdette strain, none with Jackson County strain. No discolorations at 20° or at 15° C. At 25° one tip of a true leaf and one coleoptile tip of another plant infected, infection being in the form of yellowish-brown discoloration extending 1 cm. downward and involving the whole width of the leaf and coleoptile, with an indefinite lighter colored border. Microscopic examination shows brownish tissue teeming with bacteria, which ooze out in thread-like whorls in a water mount.

At 30° C., pot sprayed with Jackson County strain showing one leaf with dark brown withered tip and with light-yellow border extending about one-half way down the leaf and farther, extending as a broad, yellow streak along the midrib. Pot sprayed with Burdette strain showing five leaves of as many plants infected. Symptoms comparable to those given above. Each leaf carefully examined under the microscope and found to harbor bacteria in the discolored and dead areas.

At 35° C., pot sprayed with Jackson County strain shows four true leaves on as many plants badly diseased at the tips and extending along the sides for several centimeters or involving most of the leaf area. Same symptoms as at 30° C. Each infected leaf examined under the microscope and bacteria found in each. All coleoptiles brown, and more or less disintegrated near the soil line. Pot sprayed with Burdette strain shows six leaves badly infected at the tips and sides. Four others with infections just beginning at the tips. No fungi were noted in the whole of the above recorded plants and the bacteria appeared as the typical short, plump rods. All check plants sprayed with water remained healthy.

December 5, 8:30 A. M.—Temperature of 35° chamber reads 33° C. Others O. K. No infections noted other than those recorded above.

December 6, 8:30 A. M.—Pot kept at 35° C. and sprayed with Burdette strain shows one seedling with dead stem, the brown water-soaked tissue extending from below the soil line to above the node, 1.5 to 2 cm. in length. Microscopic examination shows tissues teeming with bacteria; no other microorganisms found. All other plants showing no stem rot.

December 7, 8:30 A. M.—Pot at 30° C. sprayed with Burdette strain shows three seedlings with brown discoloration at the base of the stem, the discoloration extending into the interior. Microscopic examination shows plenty of bacteria in the tissues; no other microorganisms observed. Pot at 30° C. sprayed with Jackson County strain shows one plant, previously wounded at the base with a knife, with brown discoloration and rotted tissue at base of stem. Microscopic examination reveals bacteria present. Wounded, uninoculated plants appear perfectly sound, with no noticeable discoloration.

These studies clearly show that temperature plays a very important rôle in the inception and development of the disease. Attention is called to the fact that both air and soil temperatures were under control in these experiments. They fully confirmed field observations and the numerous other trials at artificial infection made in the field and in the greenhouse, in which it was found that no infections occurred unless the temperature was fairly high.

This naturally leads to the question as to direct effect of temperature on the growth and health of disease-free corn and on the pathogene itself. A number of investigators have studied the effect of temperature on corn, particularly on seed germination and on seedling development. The careful work of Lehenbauer (10) and of Dickson (7) is especially worthy of note. The former made an intensive study of the effect of temperature on the growth of corn seedlings, using Dr. Hottes' control apparatus. He finds that the optimum temperature for the growth of shoots of maize seedlings in water culture for a 12-hour period is 32° C. (89.6° F.). At high tem-

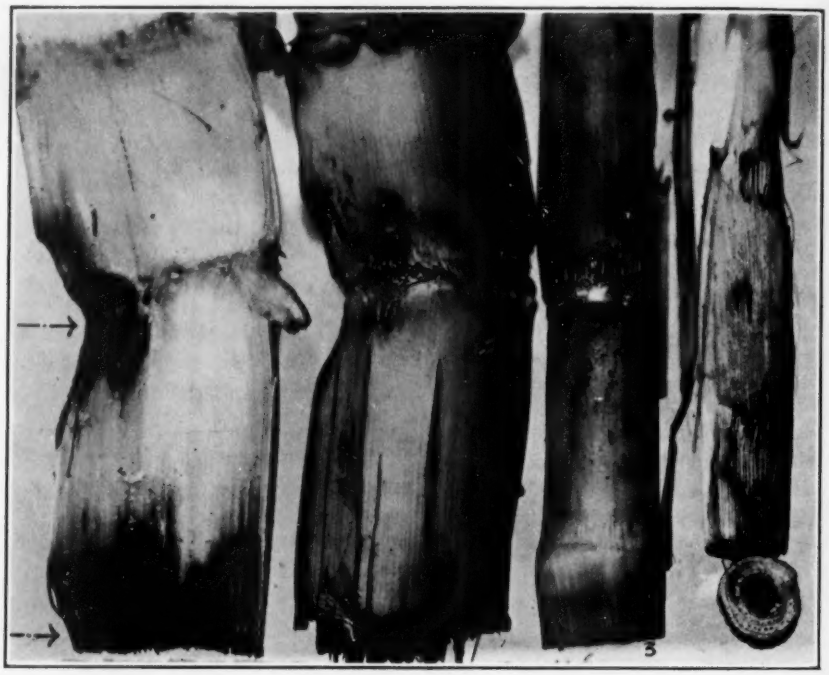

FIG. 4.—Arrows at extreme left point to rotted areas, internal view, followed by external view (to the right). No. 3 represents a nodal lesion which has not progressed to any extent into the interior. Extreme right hand figure shows spots on sheath.

peratures, 31° C. and above, the growth of these shoots is not maintained, there being a marked falling off in the rate during prolonged periods of exposure. He also finds that there is no such thing as an optimum temperature for corn seedlings, but rather a time-temperature relationship, and states that "the optimum temperature for growth under these conditions is found to change as the length of the period of exposure is altered." The growth rate at medium temperatures accords with the van't Hoff law, show-

ing a doubling of the rate for each rise of 9° or 10° C. Dickson finds that corn seed germinates more rapidly at 32° C., seedlings develop most vigorously and most uniformly at the soil temperatures of 24° to 28° C. Corn matures faster at comparatively high temperatures, the floral parts developing first at about 28° to 32° C., while at soil temperatures below 16° C. he was not able to bring corn to maturity. In general, he finds that corn functions best at rather high soil temperatures. While there does not appear to be a close agreement between the results of Lehenbauer and those obtained by Dickson, this is, at least in part, to be explained by the differences in treatments and in standards of measurement, one using both soil and air temperatures and the other using primarily soil temperatures. For the present, in the absence of further work, it may be assumed that, other factors being favorable for good growth of corn, comparatively high soil and air temperatures are best suited to its vegetative vigor and, perhaps, productiveness.

In order to determine the effect of temperature on the growth of the pathogene, several nutrient agar slants were inoculated with fresh cultures of the two different strains and placed, in duplicate, in the 20°, 25°, 30°, and 35° chambers used at the same time for one of the artificial infection series. At the end of 18 hours there was good growth at 35° and at 30°, noticeably less at 25°, and very markedly less at 20° C. As will be shown more fully later on, the organism grows very rapidly under suitable conditions.

It appears then that temperatures that are most favorable for the growth of corn are also most favorable for the growth and infectivity of the pathogene. Field evidence supports these greenhouse and temperature chamber studies. In all the more or less serious outbreaks of the disease it has been found that the weather was very warm and moist during the attacks; and conversely, in the absence of such weather, very little of the disease has been noted. Associated with this, the lower latitudes found in the eastern part of the state, particularly along the Mississippi River, in contrast to the western or hilly section, showed more frequent outbreaks and the disease was present in its worst forms, *i.e.*, complete rotting of a localized portion and a subsequent falling over of the stalk.

While temperature undoubtedly plays a very important rôle in the development of this disease, humidity, as already indicated, apparently plays just as important a rôle. While no experiments have been conducted with adequate controls to substantiate this statement, all the observations on artificial and natural infections tend to show it to be true. United States government records of precipitation and humidity of Mississippi County, Arkansas, for August, 1920, are of interest. (This was the locality and period in which 10 to 30 per cent of the corn was found ruined.) Only 6

clear days are reported for the whole month, while 5 days are listed as partly cloudy, 20 days cloudy, and 5 days with precipitation. Although the total amount of rainfall was only 4.5 inches, being 1.54 inches more than normal, the large number of cloudy days is perhaps of greater significance. Numerous comparable records could be cited which clearly show that a high humidity is indispensable. For example, the very best artificial infections, *i.e.*, those closely resembling natural stalk infections of the severe form, have been obtained in the field only when rainy weather set in immediately after the plants were inoculated. This was particularly noticeable during the last days of June and the first few days of July, 1925 (Fig. 3). Under greenhouse conditions, on the other hand, in which corn does not make as

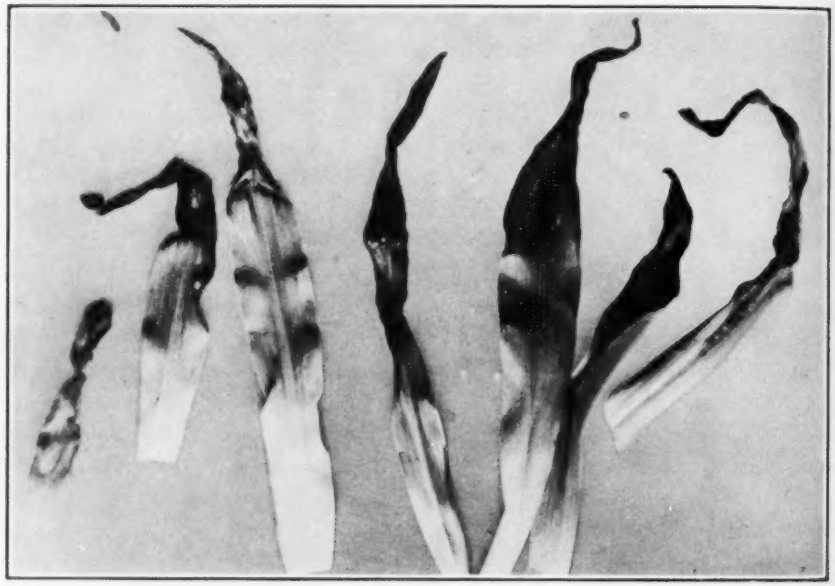

FIG. 5.—Artificial infections on leaf blades in form of dark- or light-brown, withered tips, inoculum in the form of a young culture suspended in water, applied as a fine spray by means of a sterilized, all-metallic artist syringe. Check plants sprayed with sterile water, otherwise treated in same way as inoculated plants, had healthy leaves.

vigorous growth as under proper field conditions, the extreme forms of stalk rot usually have not been observed, although infections were to be had. The explanation for this appears to be something more than the necessity of moisture for bacterial invasion, since plants covered with bell jars for 48 hours or more have been kept with moist leaf surfaces and with little or no extreme stalk rot developing. Occasionally severe infection has been obtained [(14), Fig. 3]. It appears likely that conditions which favor suc-

culence of the host, in which a comparatively large amount of soft, parenchymatous tissue and a small amount of hard woody tissue is being formed, are the direct factors which predispose a plant to infection and extreme necrotic developments. In this respect the well-known fire blight of pears may be cited as a disease comparable to the bacterial stalk rot of corn.

PATHS OF INVASION

In artificial inoculation experiments with seedlings, infections often appear on the leaf blades in which the inoculum is applied as a spray. This is to be expected, as very little actual stalk surface is exposed. Very often these infections start at the apex, indicating entrance of the pathogene through the apical hydathode (Fig. 5). Microscopic examination of such infections invariably reveals the pathogene in great numbers within the infected tissue. In addition to this form of invasion, isolated spots and streaks of blades and sheaths indicate other paths, *i.e.*, stomata or wounds. Invasion by means of leaf stomata is apparently rare, although it occurs at times. In contrast to this, when environmental conditions were favorable, the author has obtained excellent infections through stomata situated in the lower portion of the stalk (Pl. XIX, b). In this region the stomata are much more common than may be supposed. Careful studies of natural infections seem to indicate that this is one of the most common ways in which the bacteria gain entrance into the stalk. In order to test this out, a number of stalks growing in the field were inoculated by means of needle smears around the crowns, care being taken not to wound the plants; these smears were then covered with moistened absorbent cotton. Other stalks were treated in like manner, with the exception that needle pricks were made and the organism smeared in and around these. The number of infections obtained by smears was fully as great as that obtained by pricks. In these experiments, also, it is worth noting that in periods of dry weather infections by either method were few.

As far as wounds are concerned, the writer has previously (15) called attention to the natural wounds common to the corn stalk at the lower nodes, when endogenous roots are pushed out. Artificial infections have been obtained in these regions (Fig. 1), and examinations of natural infections have shown a number of cases where infections appeared to have started in tissue ruptured by the passage of one or more roots. Early infections of this sort appear as light-brown water-soaked areas of the leaf sheath and of the adjoining stalk tissue surrounding the root. Injuries caused by various insects also have been found to offer excellent paths for infection.

A histological study of diseased tissue reveals discolored cells which, depending upon the age and progress of infection, show various stages of disintegration. In young infections there is little disintegration, the bac-

teria being found within the cells and in the surrounding intercellular spaces. In older infections, however, many of the parenchymatous cells are more or less completely broken up and disjointed, and only remnants of tissue may be observed with cells appearing more or less intact. The extreme rotting and loss of cell cohesion makes the disease comparable to the soft rot of vegetables due to *Bacillus carotovorus* Jones or to the Rhizopus rot of various fruits and vegetables; a study of the enzyme action of this pathogene in all probability would reveal enzymes that are capable of acting upon pectic compounds.

THE PATHOGENE

Morphology. The organism is a short, plump, rapidly growing rod (Pl. XIX, c). (As the organism is a marked acid and gas producer and has other characteristics of *Bacillus coli,* it seemed desirable to compare these in various ways, and the results of such comparisons will be presented.) At temperatures of 25–30° C. growth occurs within 12 hours on suitable media, while in 24 hours profuse, heaped up, colonies usually appear on agar slants or on plates. With the exception of one glaring error, the brief description previously given (16) for this pathogene characterizes it quite well. The statement that it is motile by means of a single polar flagellum is incorrect and was based on stains made according to the Van Ermengem flagella staining method (Pl. XIX, a). By this and other methods, such as the Pitfield's, long streamers are often obtained, drawn out from one end of the organism. Subsequent studies enable me to say that these threads represent a slimy matrix or sheath surrounding the organism, which, when spread out on a cover glass, may be observed as masses of intertwining, coalescing threads and ramuli, which surround one or more organisms, frequently simulating flagella-like processes. As most if not all of the well-known flagella staining methods engender deposits of greater or less amounts of precipitate on and around the microorganisms, often making it impossible to obtain a clear view of them, it is not very surprising that this slime should have been interpreted as flagella. It will be shown elsewhere by the writer that at least in one other bacterium, supposedly one of the best known, the flagella have been incorrectly described. The fact that this organism in pure culture never appears motile, when observed in a hanging drop, constitutes good supplementary evidence. On the other hand, one of the reasons for the earlier belief of motility was due to the presence of actively motile bacteria, along with non-motile forms, in some natural infections. These are now regarded as secondary invaders. However, studies of flagella on a fairly large number of diverse bacteria, in which considerable variation has been observed in number and arrangement of flagella in the same species, and also the variations obtained in physiological processes

of different strains of the same species, are sufficient to make the author hesitate about any generalizations concerning motility in all possible strains. For the present, the writer can report that, in all strains of the stalk-rotting organism studied thus far (about 25 in number, but representing only two states, Arkansas and Illinois), no motility has been observed in pure cultures.

As previously recorded, the average measurements of the bacteria stained by ordinary methods are $0.7-1.2\mu \times 0.5-0.9\mu$. The organism is frequently found in groups or clumps—doubtless due to the slimy envelopes—often in pairs, and rarely in chains. Capsules are present (Pl. XIX, d) as may readily be observed by using the Plimmer and Paine method (13). By this method, including the enveloping capsules, the average measurements are $1.5-1.8\mu \times 1.0-1.3\mu$. No spores have ever been observed. Many young and old cultures have been variously stained, with no indications of spores. As the pathogene takes the form of rather small sized rods, spores would perhaps not be expected. It is Gram negative, using the method described by Burke (4). *Staphylococcus aureus* with the same stain gives a positive test. Counter-stained with safranin, the corn organism stains red, while *Staphylococcus* remains deep blue. It is not acid fast. A broth culture, 24 hours old, stained with Ziehl's carbol-fuchsin and then treated with 20 per cent sulphuric acid for a moment, shows no color. When counter-stained with aqueous methylene blue, the organism appears blue with no trace of red.

CULTURAL CHARACTERS

Agar plates. On peptone-beef agar poured plates kept at 25° C., many of the colonies become visible in 24 hours, and in 48 hours most, if not all, are visible. They are white, round, smooth, glistening, opaque, have a consistency of melted butter or of boiled starch; the elevation is convex, edge entire, and the structure amorphous. Deeply imbedded colonies, next to the glass, are flat, spreading, and have a tendency to form concentric rings. When held up to a bright light, the colonies appear brilliantly fluorescent. After three weeks, when the medium is well dried out, colonies show a marked tendency to form a flat, spreading growth, zonated near the margin, the margin noticeably lobed and scalloped.

Agar stroke.—At 25° C. there is a good growth in 24 hours, reaching a width of 4 to 5 mm. at the base of the slant, and tapering upwards. The growth is filiform to spreading (Pl. XVIII, b), raised or convex, glistening, smooth, butyrous, and opaque. (One strain isolated from Burdette, Arkansas, showed a brownish color developed in most of the common media.) Odor is marked in some strains and less evident in others, resembling putrid decaying matter.

Agar stab. Growth occurs along the entire length of the stab, somewhat better above; gas bubbles produced along the line of puncture (Pl. XVIII, c) and at times extending out through entire thickness of the medium. As a result the medium becomes cracked. Growth finally occurs in the pockets made by the gas bubbles. By suspending pieces of filter papers soaked with Nessler's reagent over cultures and heating, a brownish color appears on the paper, indicating that these gas bubbles were probably ammonia.

Gelatin plates. Growth at 17–20° C. is rather slow and not copious; colonies are round, white, with entire margins. There is no liquefaction.

Gelatin stabs. At 15–20° C. growth is observable down the whole length of the stab at the end of three days. There is no liquefaction at the end of five weeks. The line of growth is filiform, and there is a tendency for gas formation (ammonia) observable as bubbles near the line of growth.

Nutrient broth. In peptone-beef broth testing pH 7, kept at 25° C., there is a noticeable clouding and a slight precipitate, but no pellicle at the end of 24 hours. In 48 hours there is a great surface growth mainly observable in the form of a ring and floccules, or compact slimy masses and streamers. Fine whitish granules are formed in most strains (absent in some); clouding gives place to a marked turbidity with numerous floccules; the sediment is abundant and rather cohesive in most strains. In many strains there is a marked putrid odor. In contrast to the corn organism, *Bacillus coli* forms a much heavier surface growth; the sediment is never viscid (at least in the strain here used) and breaks up into compact flaky particles.

Nutrient broth over chloroform. As compared with growth on the same medium in free air, there is no clouding in 24 hours, although the precipitate is about the same. In 48 hours there is no surface growth, and the granules are not nearly as abundant as in free air; the sediment is just as heavy but more compact. At the end of six days, growth still appears considerably restricted.

Nutrient broth plus alcohol. Absolute alcohol was added to nutrient broth, so as to make 2, 3, 4, 5, 6, 7, 8, 9, and 10 per cent solutions. Growth occurs in 2, 3, 4, and 5 per cent solutions, with a heavy surface pellicle, something which does not occur in ordinary broth. At the end of three weeks, when considerable alcohol has evaporated, surface growth occurs in all up to 10 per cent, indicating that, while the alcohol at higher percentages may have considerably retarded growth and perhaps killed some of the organisms, it did not kill all the organisms at the concentrations used.

Sodium chloride in nutrient broth. At the end of three days, growth appears to be equally good in concentrations up to 3 per cent. At 4 and 5 per cent there is very little growth.

Loeffler's blood serum. Growth is restricted and whitish. There is no liquefaction.

Lima bean agar slants. Growth in 24 hours is very heavy, superior to that on nutrient agar in the same time interval, the greater growth being even more noticeable at the end of 48 hours. On this medium, growth is more spreading, less opaque, and there is a greater tendency for gas production. The medium was made up according to the directions given by the writer in a previous publication (17, p. 369).

Uschinsky's solution. Growth is good; fluid is not rendered viscid.

Fermi's solution. No growth at the end of five days.

Potato cylinders. At 25° C. there is good growth in 24 hours. It is quite heavy, heaped up, creamy-white, glistening, and not slimy. Growth is best on the moist part of the medium at the base of the cylinder. *B. coli* shows a similar type of growth but is somewhat darker in color, grayish white and flatter, with a greater tendency to spread. After the corn organism had grown on the cylinders for about a month, the cylinders were macerated, 10 cc. of distilled water added, and 1 cc. of an alcoholic solution of iodine. A purplish red (very near dusky auricula purple of Ridgway) immediately developed, indicating a partial starch hydrolysis. Cylinders upon which *B. coli* had been grown for the same length of time, and which were treated the same way, developed a deep blue color, comparable to the color of uninoculated and similarly treated cylinders.

Nutrient broth plus 1 per cent potato starch. Growth is very heavy at the surface in the form of an adhering pellicle. At the end of 11 days it was tested for starch hydrolysis by the same method given for potato cylinders. A purplish blue color appeared immediately, which gradually changed to a reddish blue, finally losing all color by standing over night. Similar treatment of growths of *B. coli* remained deep blue. This test and the one on potato cylinders indicate that the corn organism possesses diastatic action.

Potato dextrose agar slants. At 25° C. growth is copious, raised, shining, and white. In some strains there is a marked viscidity, the growth pulling away from the medium in thread-like fashion when touched with a loop. No such sliminess was noted in the strain of *B. coli* studied. In 48 hours there is a noticeable production of gas in the corn organism and much more in that of *B. coli.*

Milk. At the end of three days at 25° C. milk begins to clear in the form of a watery, honey-yellow band appearing at the surface. Coagulation is delayed, but becomes quite marked by the sixth day; color of medium is not changed. At the end of the same period cultures of *B. coli* extrude whey, which remains white and does not clear. On the 18th day, upon the addition of methyl red, cultures of the corn organism show but very faint pink, while *B. coli* cultures show a pink of around pH 5.2.

Litmus milk. There is no change in color up to the third day, aside from the watery, honey-yellow surface band, but by the sixth day the lavender color has changed to a pinkish-cream, indicating acid production. Testing with brom cresol purple, with colorimetric comparisons, the active acidity is about pH 5.4.

Indol production. On the 10th day, using the Ehrlich test, nutrient broth gave a positive test for indol. Tests by the sodium nitrate-sulphuric acid method were negative. It is of interest to note that in broth containing sodium chloride there is a decreasing amount of pink in the Ehrlich test as the percentage of salt is increased, being very markedly pink in 1 per cent and only faintly so in 5 per cent. This would seem to indicate that moderately strong solutions of table salt, and probably other inorganic salts, either hinder the production of indol or prevent its detection.

Development of crystals. In old cultures in nutrient broth containing 1 per cent dextrose, there is a noticeable development of crystals. These appear similar to those figured by Smith (19, Fig. 57, No. 2). Tests for tyrosin with Mellon's reagent were negative; likewise tests for tryptophane with bromine vapor gave negative results.

Nitrite production. Growth is fairly abundant within 48 hours in nitrate broth. Some strains develop a compact, viscid sediment. Surface growth is only noticeable as a faint ring. Tests for nitrites with starch water-potassium iodide on the 10th day are positive. Checks gave no tests.

Hydrogen-ion concentration in relation to growth. Nutrient broth was rendered acid and alkaline by adding hydrochloric acid and sodium hydroxide aseptically after the broth had been sterilized. The following series (4 tubes of each) was used: pH 3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0, 7.5, 8.0, 8.5, and 9.0. Growth occurred in the series, beginning with pH 5.0. Below that, at the greater hydrogen-ion concentration, there was no growth. It appears that the organism has a rather wide range with reference to hydrogen-ion concentration and is especially tolerant of alkaline conditions.

Toleration of acids. The following acids were added aseptically to nutrient broth after it had been sterilized: acetic, citric, hydrochloric, lactic, oxalic, and phosphoric. The concentration used for each was 0.1 per cent. The resulting hydrogen-ion concentration was: acetic acid pH 4.9, citric pH 5.0, hydrochloric pH 4.8, lactic pH 4.7, oxalic pH 4.7, phosphoric pH 4.5. Growth occurred only in the citric acid broth, testing pH 5.0. It would take much more work to show whether this means that the corn organism is intolerant of anions other than citrate, or whether the lesser concentration of hydrogen-ions in the citric acid broth was responsible for the growth.

Hydrogen sulphide production. Filter paper saturated with lead acetate hung over cultures in nutrient broth, and in nutrient broth plus 1

per cent potato starch failed to show any color, indicating no hydrogen sulphide production. However, the writer is not satisfied with this test. The recent one recommended by the committee on pure culture studies, Society of American Bacteriologists (5), has not been tried.

Ammonia production. Cultures several days old, in peptonized beef broth testing pH 7, respond readily to a test for ammonia (filter paper saturated with Nessler's reagent). The odor of ammonia is not detected however, possibly because of the very marked putrid odor produced by some strains of the organism.

Gas production. In the presence of carbohydrates the organism is a marked gas producer. This organism also produces gas in the absence of carbohydrates, but this gas, ammonia, is the product of protein decomposition. Gas production in fermentation tubes has been carefully studied, and the results are presented in table 1.

TABLE 1.—*The production of gas by Phytomonas dissolvens growing in fermentation tubes in the presence of carbohydrates*

Carbohydrate	Relative amount of gas in closed arm after 24 hrs.	Amount of gas in per cent after 5 days	Proportion of H to CO_2
Dextrose	Abundant	60 (25)[a]	1:1, (2:1)[a]
Galactose	Noticeable	15	1:1
Mannite	Noticeable	60	1:1, (2:1)[a]
Sucrose	Noticeable	60 (50)[a]	1:1
Maltose	Abundant	50 (30)[a]	1:1, (2:1)[a]
Lactose	Noticeable	25	1:1
Raffinose	Noticeable	25 (25)[a]	1:1
Inulin	Absent	Little in 5 days; 25 per cent in 10 days	
Glycerin	Noticeable	20 (30)[a]	

[a] Figures in parenthesis refer to *B. coli* cultures which were run at the same time.

Acid production. Lactose agar stabs containing Andrade-Penny's indicator kept at 25° C., develop a pinkish color in 18 hours, with considerable gas production in the form of bubbles. The pink color denoting acid development remains after two months of growth. In order to ascertain the active acidity developed by the corn organism in the presence of different carbohydrates, 1 per cent of each of the carbohydrates listed in table 2 was added to the nutrient broth. The acidity was determined by colorimetric comparisons, using the Clark and Lubs indicators.

From table 2 it will be seen that considerable acid is produced in two disaccharides and in one polysaccharide; otherwise the acid production is

not very great. Unfortunately, in the series just presented, no comparisons were made with *B. coli.* However, in a series of agar slants containing litmus as an indicator, and in the presence of various carbohydrates, the original blue was changed to red in six hours by *B. coli,* while the corn organism cultures remained blue, or showed only a mere trace of red or pink. These consisted of the following: galactose, mannite, sucrose, lactose, raffinose, and glycerin. In 24 hours, however, all the carbohydrate media were red—the corn organism cultures as well as *B. coli* cultures. It thus appears that *B. coli* develops a greater acidity in a shorter interval of time than the corn organism.

TABLE 2.—*The acid production of Phytomonas dissolvens in the presence of carbohydrates*

Carbohydrate	Original pH	pH at end of 11 days
Dextrose	6.8	6.0
Galactose	6.8	6.0
Mannite	6.8	6.0
Sucrose	6.8	5.4
Maltose	6.8	5.4
Lactose	6.8	6.1
Raffinose	7.1	6.1
Potato starch	6.8	5.4
Check (nutrient broth)	7.0	7.0

Germicidal action of mercuric chloride. Comparatively equal amounts of the organism, growing in bouillon, were transferred to tubes, each containing 10 cc. of mercuric chloride of the following dilutions: 1:1000; 1:10,000; 1:50,000; 1:100,000; and 1:500,000. At the end of five minutes a drop of each dilution was transferred to a tube of nutrient broth. No growth occurred in the following: 1:1000; 1:10,000; 1:50,000; while the transfers from 1:100,000 and 1:500,000 grew in 24 hours. After 15 minutes exposure, transfers from 1:100,000 failed to grow, while those from 1:500,000 appeared unaffected. It appears, then, that mercuric chloride is a very efficient germicide for this organism.

PHYSIOLOGICAL REACTIONS

Effect of temperature on growth. In connection with the artificial inoculation experiments, attention has already been called to the fact that this organism grows best at around 30° to 35° C. In addition to the study there reported, the organism grown on nutrient broth was subjected to various temperatures below 20° C., resulting in very poor growth or no growth at all. At 15° C. there was a slight clouding in five days; at 10° C.

there is a very faint cloud at the end of 8 days; while at 0° C. there appears to be no growth. Due to lack of facilities, the upper limits of growth have not been determined. Based on records of other bacterial pathogenes, this would probably be about 40° C., with the death point several degrees above that. In reality, such studies as these here reported, and in common use, are of little significance as to the effect of temperature under natural conditions, because, under these conditions, constant temperatures almost never exist. It would be far better to subject the organism to field temperatures at different times of the year.

Effect of drying. Diseased material collected during July of 1921 was kept in a dry condition in the laboratory until late summer and then placed near an open recess in a building where it was sheltered from rain but more or less subject to outdoor temperatures. Here it was left until the first week in July, 1922. A portion was then thoroughly washed, macerated in sterile water, and the suspension sprayed on one pot of corn seedlings. Typical infections resulted particularly in three seedlings that had been wounded. (Wounded plants which were not inoculated remained healthy.) The infected portion of one of the plants was then washed, surface sterilized, macerated in sterile water and plated out. Notes read that in the series of 10 plates, pure cultures were obtained without a single evident contamination. Transfers from the pure culture isolations were in turn inoculated on another group of plants and resulted in two typical infections on as many plants. From one of these the pathogene was reisolated. It thus appears that the organism may readily live over for a year at least in dry tissue.

Overwintering. The above data also indicate one method by which the pathogene may live over winter. As the primary seat of stalk infection is close to the crown of the plant, the diseased part has an excellent opportunity of remaining undisturbed during harvesting of the corn, whether or not the stalks are cut. The stubble remaining on the field serves as a source of infection for next year's corn if no rotation is practiced. Experimental evidence for other means of overwintering unfortunately is not at hand, due to the destruction of inoculated ears on three different occasions. Field data gathered at various places seem to indicate that the pathogene may also be carried in, or on, seed. For example, one field was cleared of timber and was used for growing corn for two years, then alfalfa for seven years, followed by another crop of corn. Upon this last crop the disease was discovered. Of course such evidence is not very satisfactory for various reasons. For one thing, ears showing natural infections have not yet been found. Although ears showing rotted husks and kernels have been frequently observed, some of which harbored bacteria in the diseased parts, all attempts at isolation have yielded either fungi or

bacteria which have no resemblance to the species here discussed. Hence the actual means of disease introduction in different localities remains to be investigated. As artificial infections sometimes are secured on leaf blades and sheaths, and as natural infections on lower sheaths are common, there is very good reason to suspect that natural infections take place on husks at least and also, perhaps, on the kernels themselves. Indeed, in one case a Golden Bantam seedling growing in sterilized soil was infected with bacterial stalk rot. The seed came from the same lot as those which produced 11 diseased hills out of 20, previously mentioned.

Effect of low temperatures and freezing. Three different experiments were undertaken to determine the effect of very low temperatures. First, freshly made strokes on nutrient agar, as well as full grown strokes, were put into a calcium chloride water mixture, operated in conjunction with an ice machine, with a temperature of — 17° C. Tubes were left in this for 10, 30, 60, 90, and 120 minutes, and then placed in a 30° C. incubator. After 24 hours of incubation, growth occurred on all the freshly made strokes, while transfers made from the full grown ones also made successful growth. Second, old strokes of two different strains growing on nutrient agar were placed in a melting ice mixture, kept there for two hours, and then placed in a 30° C. incubator. All transfers made from these grew. Third, a loopful of a 48 hour broth culture was placed in each of four tubes containing the same quantity of nutrient broth. Three of the tubes were placed in a freezing brine ice mixture, in which the broth solidified, and was kept in a frozen solid state for two hours. After thawing, poured plates were made from similar quantities of each of the three tubes, as well as from the check. Counts of the number of colonies developed from check as well as from the frozen cultures showed a reduction of about 90 per cent of the colonies in the frozen cultures. Considering the differences in media and in type of treatment, these three experiments are not as conflicting as they appear. While the mortality of the organism in a liquid medium subject to extreme freezing is high, it can, as a whole, withstand very low temperatures.

Effect of sunlight. Poured plates placed on a block of ice in bright sunlight (mid-day of the 22d of June), with one-half of each plate covered with black paper, were exposed for varying periods of time. Those exposed for 30 and 45 minutes showed no difference in number of colonies between exposed and unexposed halves. But those subject to one hour exposure and over were almost entirely killed. Compared to other bacterial phytopathogenes, a number of which are said to succumb after exposure of 15 to 30 minutes, the corn organism is not so sensitive to sunlight.

Vitality on culture media. On ordinary media such as nutrient broth, nutrient agar, and on milk, the organism lives for quite a few months. For

example, nutrient agar stroke cultures which were kept in an icebox for 10 months, during which time the medium dried out considerably, although not losing all moisture, showed growth in 24 hours when transferred to nutrient agar.

Group number. Using the most recent chart (1925) issued by the Society of American Bacteriologists (5), the index number is 5000–32021–1111.

RELATIONSHIP OF THIS DISEASE TO BURRILL'S BACTERIAL DISEASE OF CORN

In the first publication (14) of this disease it was stated that it "has been known since 1889" and that it was first described by Burrill. This statement was based upon Dr. Burrill's description of the disease (2 and 3) and not upon any specimens seen by the writer. Having now made a very complete study of all the specimens left by Burrill at the University of Illinois, the present author can now report that it is very doubtful if the disease or diseases Burrill dealt with is the one here considered. Most, if not all, the material examined showed various blade and sheath spots (Pl. XVII, e), quite comparable to the spots described by Durrell (8 and 9). Only one cornstalk specimen was found, representing a seedling, and in this the symptoms are markedly different from the bacterial stalk rot. Microscopic examination of a number of these spots show various fungi and bacteria present within the tissues, confirming the gross symptoms as to type of disease. All the collections examined, including those having the following numbers, show the ordinary sheath spots: 9153, 10527, 25896, and 32392.

Aside from the question as to whether the disease described by Burrill is the one here discussed, Burrill described, but apparently did not name, the causal organism, although various well-known authors, including Russell (18, p. 36), Migula (12, p. 315), Ludwig (11, p. 95), Voglino (21, p. 37), and quite a few of the more recent authors of text-books attribute both *Bacillus zeae* and *Bacillus secales,* the latter supposedly a synonym, to Burrill. Most of these writers cite Burrill's publications on this subject, and when these are carefully studied it appears very doubtful if the authors ever read Burrill's work. He has only two articles on the corn disease that are of importance (2 and 3) and several others that are merely abstracts and reviews. In none of these does he give any name. The earliest use of the name *Bacillus zeae* Burrill that the present author has been able to find appears in Russell's work (18) in 1892, and it would not be surprising if that author, who was working on practically every bacterial plant pathogene then known and on other types of bacteria, for convenience adopted a name, and used for authority, the name of the man from whom he had obtained his cultures. Certainly, as late as 1891 Burrill had not

given a name to the organism, for on package No. 10527 of Burrill's material is found the following data: "A bacterial disease of corn, described by Burrill but not named. On Zea mays, Urbana, Ill., June 17,1891. C. P. C(linton) Det. McCluer, Coll." This date is so close to the date of Russell's publication, 1892, that the explanation offered above for the origin of the name, or rather two names, seems plausible.

The difference between the disease here discussed and Stewart's disease has previously been presented (14).

TECHNICAL DESCRIPTION

Phytomonas dissolvens (comb. nov.)[5]
(Pseudomonas dissolvens Rosen.)

Non-motile, short, plump rods, 0.6 by 1.0μ; facultative anaerobe, acid and gas produced on various carbohydrate media; no spores; capsules present; colonies on nutrient agar plates white, round, entire, glistening, opaque, some strains emitting a strong putrid odor; nitrates reduced to nitrites; ammonia produced; indol produced; hydrogen sulphide not produced (?); diastatic action marked; fair growth in Fermi's and Uschinsky's solutions; optimum temperature for growth 30°C.; vitality on culture media comparatively long; gelatin not liquefied; somewhat sensitive to drying, freezing, and sunlight; Gram negative; not acid fast. Pathogenic on *Zea mays* L.

SUMMARY

The bacterial stalk rot of corn has been reported from Arkansas, New York, North Carolina, Mississippi, Louisiana, Ohio, Illinois, North Dakota, and Arizona.

The disease has been noted in Arkansas for 6 years and is considered to be serious during those periods of the corn-growing season when the rainfall and humidity are above normal. In the absence of high temperatures and humidity the disease is found to be entirely absent or of minor importance.

[5] If one considers the aim of classification to consist primarily of bringing together and grouping into genera all forms that are morphologically related, then the recent proposals brought out by the Society of American Bacteriologists (1) will be condemned; but if the similarity of physiological processes, rather than morphology, be considered the guide, then the new proposals will be accepted. It is the humble opinion of the writer that so little is actually known about the morphology as well as physiology of the supposedly well known organisms, that almost any system which would be proposed is certain to be full of errors. The important thing for the present is agreement among bacteriologists on one system; otherwise, chaos in nomenclature is sure to rule. The recently proposed genus *Phytomonas*, therefore, is being accepted. If Migula's classification is followed, the combination should be *Bacterium dissolvens* (comb. nov.); and if Erwin F. Smith's classification is used, the combination *Aplanobacter dissolvens* (comb. nov.) is suitable.

It is described as primarily affecting the stalks and leaves. Symptoms are as follows: (a) a light- or dark-brown rotting of bases of leaves, particularly those at the base of the stalks; (b) a rotting of the lower portion of the stalk, the affected part being dark-brown, soft, putrid, and sunken in fresh infections. These may extend clear through the entire width; or, as more frequently found, remain localized as dark, rotted spots with margins appearing water-soaked. The disease consists of a localized necrosis of parenchymatous tissue.

Attacked tissue is found teeming with bacteria, and more than 50 isolations are reported from such infections. Many successful artificial infections are also reported, including infections obtained in Hottes chambers in which accurate control of soil and air temperatures are obtainable. It is found that temperature plays an important role in disease inception and development. Below 20° C. no infections take place; between 20° and 25° C. infections are few; between 30° and 35° C. the greatest number of infections result. High humidity is also found essential for infection. A correlation is noted between rapid succulent growth and disease susceptibility.

Infections are found to take place through hydathodes, stomata, and through injuries resulting from the extrusion of endogenous roots, and from insects.

The pathogene is described as a short, plump, rapidly growing rod, white in most culture media, and is considered to be closely related to *Bacillus coli*. Numerous cultural and physiological reactions are described. The pathogene is named *Phytomonas dissolvens* (comb. nov.).

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LITERATURE CITED

1. BERGEY, D. H. Chairman and others. Manual of determinative bacteriology. Williams and Wilkins, Baltimore. 1923.
2. BURRILL, T. J. A bacterial disease of corn. Ill. Agr. Exp. Sta. Bul. 6: 165–175. 1889.
3. ———. A bacterial disease of Indian corn. Soc. Prom. Agr. Sci. 7: 19–27. 1889.
4. BURKE, VICTOR. Notes on the Gram stain with description of a new method. Jour. Bact. 7: 159–182. 1922.
5. COMMITTEE ON BACTERIOLOGICAL TECHNIC OF THE SOCIETY OF AMERICAN BACTERIOLOGISTS. Manual of methods for pure culture study of bacteria. Geneva, New York. 1923–1925.
6. DAWSON, W. J. Annual report of the mycologist for the year ending 31st March, 1918. Dept. of Agr. British East Africa. Ann. Rept. 1917–1918: 123–140. 1921.

7. DICKSON, JAMES G. Influence of soil temperature and moisture on the development of seedling blight of wheat and corn caused by *Gibberella saubinetii*. Jour. Agr. Res. **23**: 837–870. 1923.
8. DURRELL, L. W. A preliminary study of the purple leaf sheath spot of corn. Phytopath. **10**: 487–495. 1920.
9. ————————. Dry rot of corn. Iowa Agr. Exp. Sta. Res. Bul. **77**: 346–376. 1923.
10. LEHENBAUER, P. A. Growth of maize seedlings in relation to temperature. Physiol. Res. **1**: 247–288. 1914.
11. LUDWIG, FRIEDRICH. Lehrbuch der niederen Kryptogamen, Stuttgart. 1892.
12. MIGULA, W. System der Bakterien. **1**. 1897.
13. PLIMMER, H. G., and PAINE, S. G. A new method for the staining of bacterial flagella. Jour. Path. and Bact. Edinb. **4**: 286–288. 1921.
14. ROSEN, H. R. A bacterial root-rot of field corn. Ark. Agr. Exp. Sta. Bul. 162: 1–7. 1919.
15. ————————. Further observations on a bacterial root and stalk rot of field corn. Phytopath. **11**: 74–79. 1921.
16. ————————. The bacterial pathogene of corn stalk rot. Phytopath. **12**: 497–499. 1922.
17. ————————. A bacterial disease of foxtail (*Chaetochloa lutescens*). Ann. Mo. Bot. Gard. **9**: 333–402. 1922.
18. RUSSELL, H. L. Bacteria in their relation to vegetable tissue. Johns Hopkins Univ. Doct. Diss. Baltimore. 1892.
19. SMITH, ERWIN F. Bacteria in relation to plant diseases. Carnegie Inst. Washington, Pub. No. 27. 1. 1905.
20. TEHON, L. R. A preliminary report of the occurrence and distribution of the common bacterial and fungous diseases of crop plants in Illinois. Natural Hist. Survey, Ill. Dept. Register. and Educ. **15**: I–VIII, 173–325. 1924.
21. VOGLINO, P. Patologia vegetale. Torino. 1905.

EXPLANATION OF PLATES

PLATE XVII

a—Natural infection at a lower node showing extreme necrosis and shrinking of diseased tissues and disjointed threads, representing remnants of the fibro-vascular bundles. Marked depression in nodal circumference due to destruction and collapse of parenchymatous tissue.

b—Artificial infections on Country Gentleman by means of needle pricks, the needle carrying pure cultures of the pathogene; infections appear as small, dark brown, or blackish streaks and spots.

c—Artificial infection on Mexican June field corn, in the form of a rotted internode, resulting from hypodermic injection of a pure culture of *Phytomonas dissolvens* (checks injected with sterile water remained healthy).

d—Natural infections on lower part of stalk, mostly in the form of localized spots.

e—Typical specimen, representing sheath infections, left by Dr. Burrill in the herbarium of the University of Illinois and labeled by him, "probably bacterial."

PLATE XVIII

a—Colonies of *Phytomonas dissolvens* isolated from a rotted node, 48 hours old, on nutrient agar. Surface colonies whitish, opaque, buried colonies are much flatter and appear lighter.

b—Growth on nutrient agar slant after 24 hours at 28° C.

c—Growth of the corn pathogene along entire length of stab in nutrient agar, with a gas bubble shown in one.

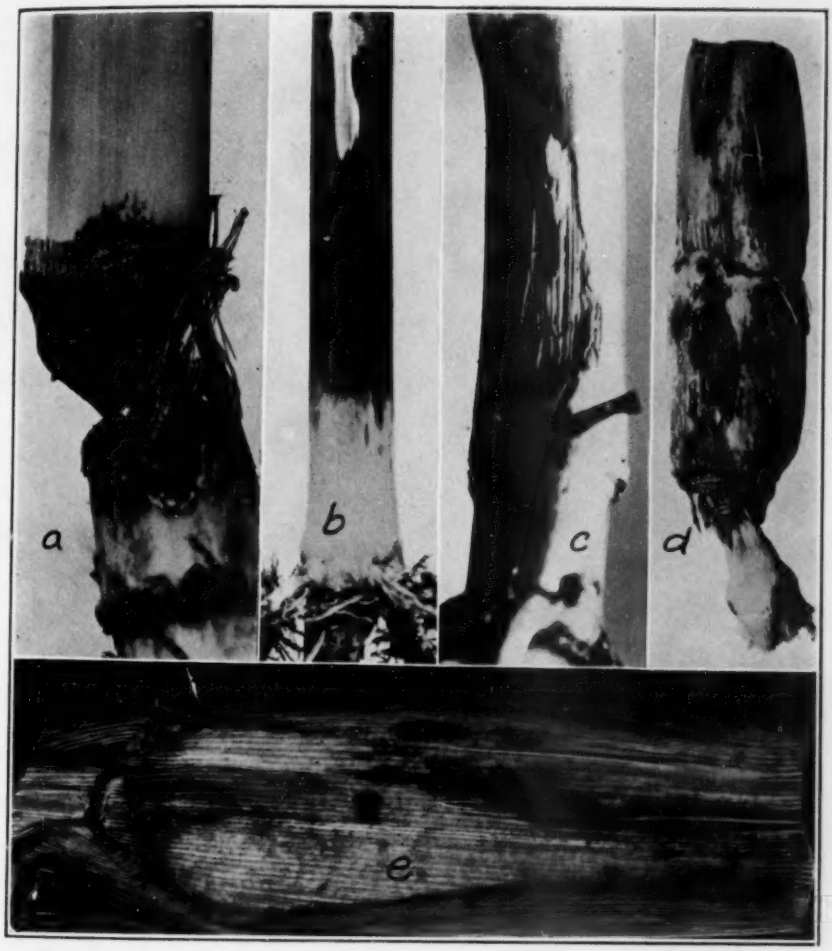
PLATE XIX

a—*P. dissolvens* stained by the Van Ermengem method for showing flagella. The streamer, connected to the rod on the extreme right, appears to be a flagellum, but, in view of the entire absence of motility in hanging drops of pure cultures, and in view of the extreme viscidity of certain strains, this and similar appearing structures are interpreted as being part of the slimy matrix surrounding the individual rods. Magnified about 1,000 times.

b—Artificial inoculation of *P. dissolvens* on Golden Bantam sweet corn, showing relationship of stomata to infection. View represents an unstained, water mount of a tangential section of the cornstalk immediately below the soil line. Note the bacteria, appearing as a dark mass, in a cell adjoining one of the stomata, the two other stomata and their neighboring cells being normal.

c—*P. dissolvens* stained with carbol-fuchsin. Magnified about 1,000 times.

d—Corn pathogene stained to show capsules. This staining enlarges the organism considerably as compared to ordinary staining. Magnified about 1,000 times.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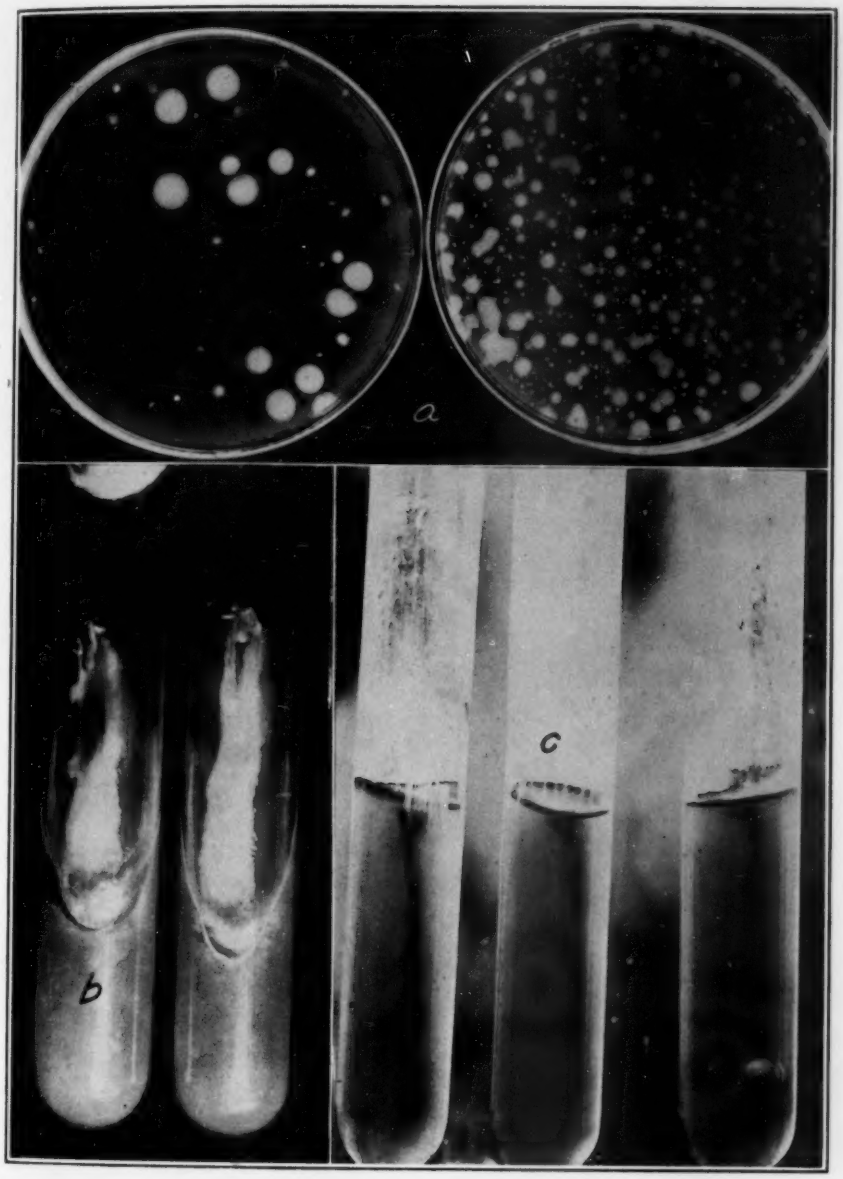
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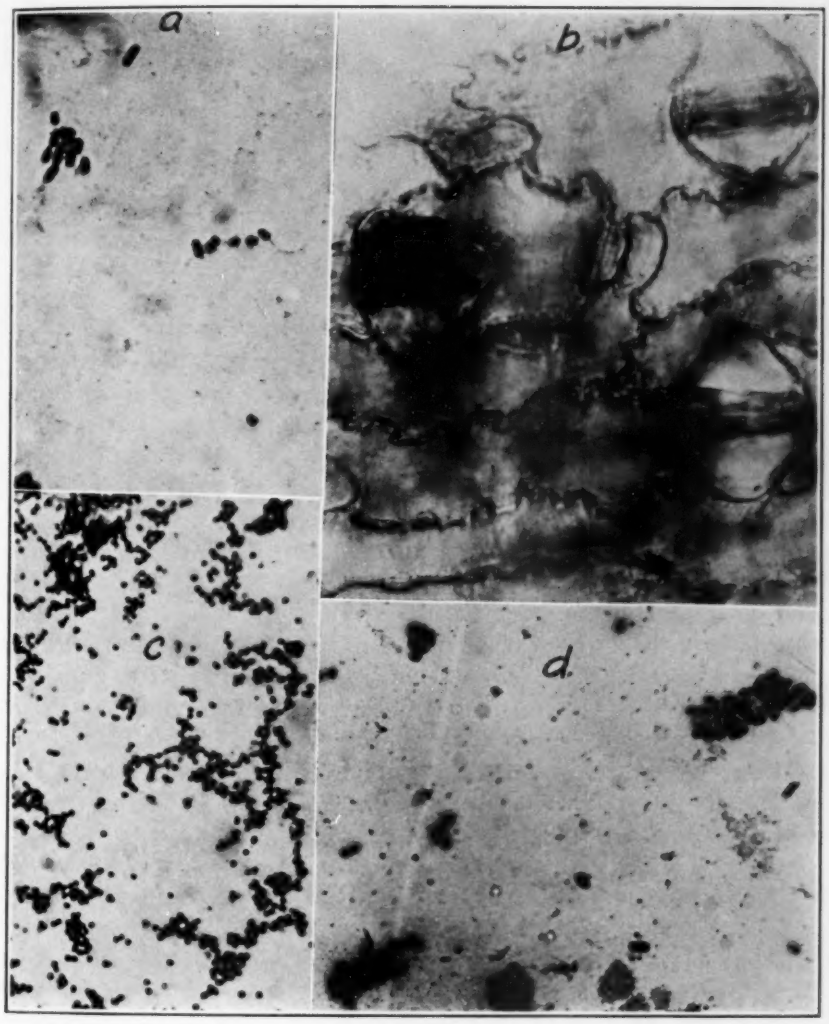
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THE NATURE OF RESISTANCE TO FOOTROT CAUSED BY ASCOCHYTA SP.[1] AND SOME OTHER FUNGI IN THE EPICOTYL OF THE PEA

GRACE G. GILCHRIST

INTRODUCTION

In the investigation by F. R. Jones (1) of the cause of failure of peas in certain parts of the United States, it was found that, in many dying plants, the base of the epicotyl and the upper portion of the taproot were decayed, sometimes to such an extent that the plant broke readily at this point. This type of injury is usually called footrot, to distinguish it from the more extensive decay of the root system called rootrot. Footrot, or decay of the base of the stem, is not due to a single parasite, but may be caused by any one of several widely different parasites upon the pea plant. Among these are *Fusarium martii* var. *pisi*, *Pythium* species, *Rhizoctonia solani* Kühn, and a species of *Ascochyta*. The fact that such a number of fungi find the base of the epicotyl, and even a limited region at the base of the epicotyl, more accessible to invasion than the upper portion of this underground structure, and more accessible than the taproot below, suggests that this region has some structural or other peculiarity which makes it easy of access to weak parasites. If the nature of this peculiarity can be discovered, and if it is found to be possessed unequally by different varieties of peas, making some more resistant to footrot than others, such finding will constitute a contribution to the studies of the nature of resistance of peas to disease, now in progress. Resistance of varieties of peas to soil inhabiting parasites which accumulate in old pea fields and which undoubtedly include those producing footrot seems to have been demonstrated by field trials in Wisconsin, as well as by several investigators elsewhere. It is the object of the present study to determine whether certain varieties resistant in field trials are resistant to the typical footrot caused by *Ascochyta* sp., and to discover the factor or factors which render the base of the epicotyl easy of access to this, and perhaps the other, parasites causing footrot.

[1] This is the "Phoma-like" fungus mentioned by Jones and Linford (2) as a cause of footrot of peas in Wisconsin. Further study of this fungus is in progress to determine its relation to *Ascochyta pisi* Lib.

DESCRIPTION OF FOOTROT CAUSED BY ASCOCHYTA

The fungus causing this disease enters the base of the epicotyl and from there spreads up and down through the cortical tissue. A dark brown lesion, triangular in shape, with the apex of the triangle pointing upward, is formed on the epicotyl. In slightly affected plants the fungus extends inward only as far as the endodermis, and there is no appreciable shrinking of the tissue. The fungus spreads downward into the taproot, but that organ is not usually seriously injured. In more severe cases the epicotyl is girdled, and there is considerable shrinkage of the tissues between the four strands of sclerenchyma, giving the injured stem an angular appearance. The taproot in this case is blackened, completely girdled, and may be severed. Here the vascular system is penetrated by the fungus. Pycnidia are found on these lesions and also on the seedcoat (Plate XX, C).

Lesions caused by this fungus are distinguished from those caused by *Fusarium martii* var. *pisi* chiefly by their color, those caused by *Fusarium* being somewhat of a reddish brown. Lesions caused by *Rhizoctonia* in this region of the epicotyl are usually more corroded and sunken in character than those caused by the *Ascochyta* (Plate XX). Lesions caused by *Pythium* species usually are sunken, but are straw colored or very light brown. Lesions produced by these fungi hardly can be distinguished by form or difference in location.

CHARACTERS OF THE EPICOTYL

The epicotyl, that portion of the stem between the point of attachment of the cotyledons and the first node, in the pea resembles the root in many respects. It produces both root-hairs and secondary roots. As a rule, however, these are formed, not at the very base of the epicotyl, but slightly above it. It is this hairless region which is most susceptible to attack by various parasites (Plate XX).

The true root possesses a definite endodermis, but immediately above the base of the epicotyl this endodermis is not entire, and, progressing further up, it becomes less and less conspicuous.

For detailed study of this region, material of the Alaska variety of pea was fixed in formal-alcohol (60 per cent alcohol—96 cc., formalin 4 cc.). Parts of the same plant, placed in the same fixative, gave varying results. Portions of the stem above soil level fixed excellently, whereas with the epicotyl the outer layers showed considerable plasmolysis, although the inner tissues were well fixed. It seemed as if the alcohol of the fixative had withdrawn water from the epidermal cells of the epicotyl more quickly than from the epidermal cells of the second or third internodes.

Transverse sections of this material stained with Sudan III showed marked differences in the amount of cuticle in the aerial portions of the

stem and in the epicotyl. In the epicotyl itself a diminution in the cuticular development was observed in passing towards its base. The difference in the amount of cutin between the top and the base of the epicotyl suggested that this might be a contributing factor in the resistance or susceptibility of various varieties of peas.

INFECTION RATING TO DETERMINE RELATIVE SUSCEPTIBILITY OF VARIETIES

Since differences responsible for resistance are more easily recognized in the comparison of similar varieties, representatives of similar growth maturing at different seasons were chosen. Alaska and Rice's No. 330 are both early varieties which mature at approximately the same time and are almost identical in appearance and habit of growth, the Rice's No. 330 differing primarily in its disease-resistant character. Saxa is also an early variety, which, owing to its great susceptibility to all pea diseases, has been discarded by commercial growers. Horsford and Horal are dwarf varieties, Horal being a Wisconsin cross between Alaska and Horsford, recently developed and introduced as a promising disease-resisting type. The White Marrowfat and Black-eye Marrowfat are old horticultural varieties and are used chiefly for garden or field culture. Future reference to the resistance or susceptibility of these varieties will be made by R. or S. These varieties were grown in the greenhouse in Wisconsin soil temperature tanks at approximately 18°C.[2] in soil which had been inoculated with the *Ascochyta*. The infection rating, according to a method described by McKinney (3), was as follows:

Susceptible varieties			Resistant varieties		
Name	Season	Per cent infection	Name	Season	Per cent infection
Saxa	early	64	Rice's No. 330	early	38
Alaska	early	42	Horal	mid-season	44
Horsford	mid-season	48	Black-eye Marrowfat	late	41
White Marrowfat	late	50			

MICROCHEMICAL TESTS

From the check plants in the above experiment, transverse sections were made of the epicotyl and stained with Sudan III for cutin. The resistant varieties, Rice's No. 330, Horal and Black-eye Marowfat, showed a definite

[2] Experiments conducted in the Wisconsin soil temperature tanks to determine the most suitable temperature for the early development of the pea plant showed that the greatest increase in weight took place at 18° C.

cuticle in the aerial parts of the stem. The reaction for cutin was less pronounced on passing towards the base of the epicotyl. However, at the level of the cotyledons, a slight amount was still present.

With susceptible varieties there was no appreciable difference in the cuticle of the aerial parts from that of the resistant varieties mentioned above, but, in passing towards the base of the epicotyl, the cuticle entirely disappeared.

This variation in the amount of cuticle present in the susceptible and resistant varieties suggested a possible difference in the rate of drying of corresponding portions of the epicotyl.

EXPERIMENTS TO DETERMINE THE RATE OF EVAPORATION FROM THE EPICOTYL

The above seven varieties were planted in sand, with soil immediately below them, and placed in a greenhouse with an air temperature of 18–20°C. At the end of seven days the plants were taken up, and the first internodes of twenty representative plants were selected. These were divided into three equal portions, base, middle and top. Records of the length and diameter of each portion were made. The latter measurement was read by means of a Zeiss micrometer gauge.

In order to avoid evaporation from the cut ends of the portions, these were dipped into melted vaseline. Weighings were made every two hours for eight hours. In calculating the per cent loss of water from the surface of each portion, it was necessary to take into consideration the weight of the vaseline. The latter was obtained by weighing a filter paper, heating it, allowing it to absorb the vaseline from the cut ends, and then re-weighing. The difference between these two weights gave the weight of the vaseline. From this data the actual weight of each portion was obtained.

Table I represents the loss of water calculated as a percentage of the original weight. It will be noted that in every variety tested there was a greater loss in weight from the base of the epicotyl than from the top of the epicotyl. Also, in comparing the similar varieties, there was a greater loss from the base of the epicotyl of Alaska than from that of Rice's No. 330; more from Horsford than from Horal; and more from White Marrowfat than from the Black-eye Marrowfat. From these results, together with the area of the evaporating surface calculated from the measured length and diameter of each portion, the amount of water evaporated from 100 sq. cm. was calculated. The results thus obtained are shown graphically in figure 1. They show that there was a direct correlation between the rate of evaporation and relative susceptibility to footrot among similar varieties. There was more evaporation from Alaska (S) than from Rice's No. 330 (R), more from Horsford (S) than from Horal (R), and more from White Marrowfat (S) than from Black-eye Marrowfat (R).

DISCUSSION

Several points of interest may be deduced from these results. In all the varieties examined, more moisture is evaporated from the surface of the lower third of the epicotyl than from the upper third. This result

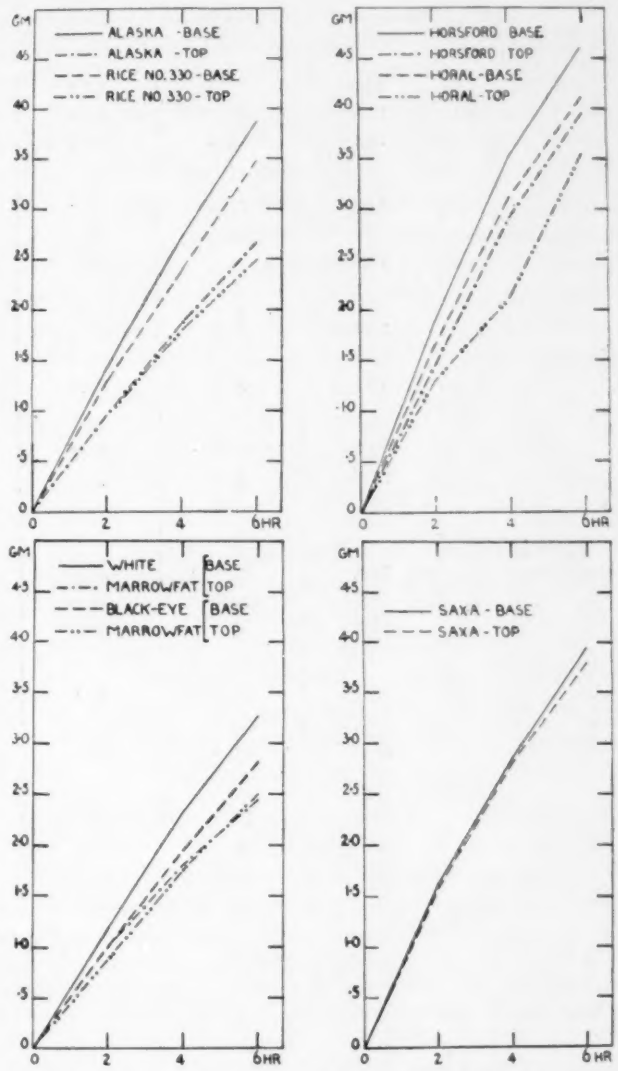

FIG. 1. Graphs representing the loss of water by evaporation at intervals of two hours. A direct correlation between rate of evaporation and susceptibility to pea footrot is shown. The rate of evaporation increases with the increased susceptibility of varieties and of the different portions of the epicotyl.

TABLE 1.—*Summarized data of the rates of evaporation of different portions of the stem on the same pea plant and also the rates shown by plants of varieties differing in relative susceptibility to fungous invasion*[a]

Variety	Water loss in per cent			
	After 2 hours	After 4 hours	After 6 hours	After 8 hours
Rice's No. 330 (R)				
Base	22.3	41.5	60.1	74.3
Middle	21.0	37.0	52.3	64.2
Top	16.7	31.0	44.1	52.0
Alaska (S)				
Base	27.3	50.9	72.4	85.5
Middle	20.4	39.6	54.6	64.1
Top	17.5	34.1	49.0	60.2
Horal (R)				
Base	25.1	46.7	58.1	77.8
Middle	27.7	51.8	69.6	80.4
Top	18.1	29.5	49.3	61.4
Horsford (S)				
Base	32.6	60.0	79.7	88.9
Middle	27.1	51.5	71.9	84.6
Top	23.2	44.4	62.8	77.1
Black-eye Marrowfat (R)				
Base	16.8	32.7	47.0	59.2
Middle	16.5	30.1	43.9	54.2
Top	13.9	28.0	39.7	50.3
White Marrowfat (S)				
Base	20.1	39.9	56.1	70.7
Middle	18.1	33.2	47.5	60.6
Top	16.4	29.3	40.0	50.0
Saxa (S)				
Base	28.4	52.4	69.9	80.5
Middle	29.3	52.4	69.6	78.2
Top	26.5	48.0	64.4	74.8

[a] Note that in all cases the rate of evaporation increases from the more heavily cuticularized top to the less heavily cuticularized base. Note also, in comparing the relatively susceptible (S) and resistant (R) varieties as paired, that there is a direct correlation between higher rate of evaporation and the relative susceptibility of the variety.

corresponds with that obtained by microchemical tests, and seems to point to the fact that the lower portion of the epicotyl is deficient in cuticle. Since cuticle consists of an unbroken lamella containing no cellulose fabric,

it forms an effective barrier against the penetration of most fungi. Consequently the lack or deficiency of it at the base of the epicotyl of the pea may account for the susceptibility of that region to numerous fungi. Again comparing Rice's No. 330 and Alaska, both early season varieties, the loss of water after two hours from Rice's No. 330 (R) was 22.3 per cent as compared with 27.3 per cent from Alaska (S); in the mid-season varieties, the loss from Horal (R) was 25.1 per cent as compared with 32.6 per cent from Horsford (S); and in the late-season varieties, the loss from Black-eye Marrowfat (R) was 16.8 per cent as compared with 20.1 per cent from White Marrowfat (S). Whether the presence or absence of cuticle is entirely responsible for susceptibility or resistance is a difficult question to answer, but the above results clearly seem to suggest that there may be an important correlation. No variety of pea has been found to be immune to footrot, but those which are more resistant possess a thicker cuticle than those which are less resistant. The base is the most susceptible portion of the epicotyl, and it is this portion which develops the thinnest cuticle. Priestley (4) states that the difference between cutin of different plants is due in part to differences in their constituent acids, and in part to differences in the external and internal conditions prevailing whilst these acids pass over into the form that they assume in the mature cutin lamella. Therefore the above results are relative, and should not be regarded as absolute. Consequently, should these experiments be repeated, the amount of water evaporated from the same varieties probably would not coincide with the above results, but the relative amount should be comparable.

In the case of the Ascochyta footrot in plants which have been attacked but recovered, the fungus appears to have penetrated at the base of the epicotyl and traveled inwards as far as the endodermis. Here it seems to have met a barrier comparable with that of the cuticle on the surface. Therefore an investigation of the endodermis in the pea plant as to its possible relation to disease resistance should give further interesting results.

SUMMARY

1. In previous investigation of the diseases of the garden pea, it has been observed that a group of parasitic fungi enter the base of the epicotyl more frequently than elsewhere in the underground stem or root. These fungi include a species of *Ascochyta* which causes a footrot of peas in Wisconsin. This paper presents the results of a search for the characteristics of this basal portion of the epicotyl which render it especially susceptible to invasion, and for possible differences in susceptibility of this region in different varieties of peas.

2. Microscopic observation and experiments on rate of evaporation have shown that the susceptible region at the base of the epicotyl is deficient or

lacking in a covering of cuticle. The middle and upper portions of the first internode show a thicker cuticle in all the varieties studied.

3. Inoculations with a species of *Ascochyta* have shown that varieties of peas described as resistant in field trials are less injured by this fungus than are the susceptible varieties. Comparison of these varieties has shown that the resistant varieties have a better development of cuticle at the base of the epicotyl than do the susceptible ones.

4. From this work it appears that deficiency or absence of cuticle at the base of the epicotyl is one of the conditions which make this region particularly susceptible to disease of the footrot type, and that varietal difference in susceptibility may be due in part to differences in the degree of cuticularization of this region.

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LITERATURE CITED

1. JONES, F. R. Stem and root rot of peas in the United States caused by species of *Fusarium*. Jour. Agr. Res. **26**: 459–475. 1923.
2. JONES, F. R. and LINFORD, M. B. Pea disease survey in Wisconsin. Wis. Agr. Exp. Sta. Res. Bul. 64: 1–30. 1925.
3. MCKINNEY, H. H. Influence of soil temperature and moisture on infection of wheat seedlings by *Helminthosporium sativum*. Jour. Agr. Res. **26**: 195–217. 1923.
4. PRIESTLEY, J. H. Suberin and cutin. New Phytologist **20**: 17–29. 1921.

DESCRIPTION OF PLATE XX

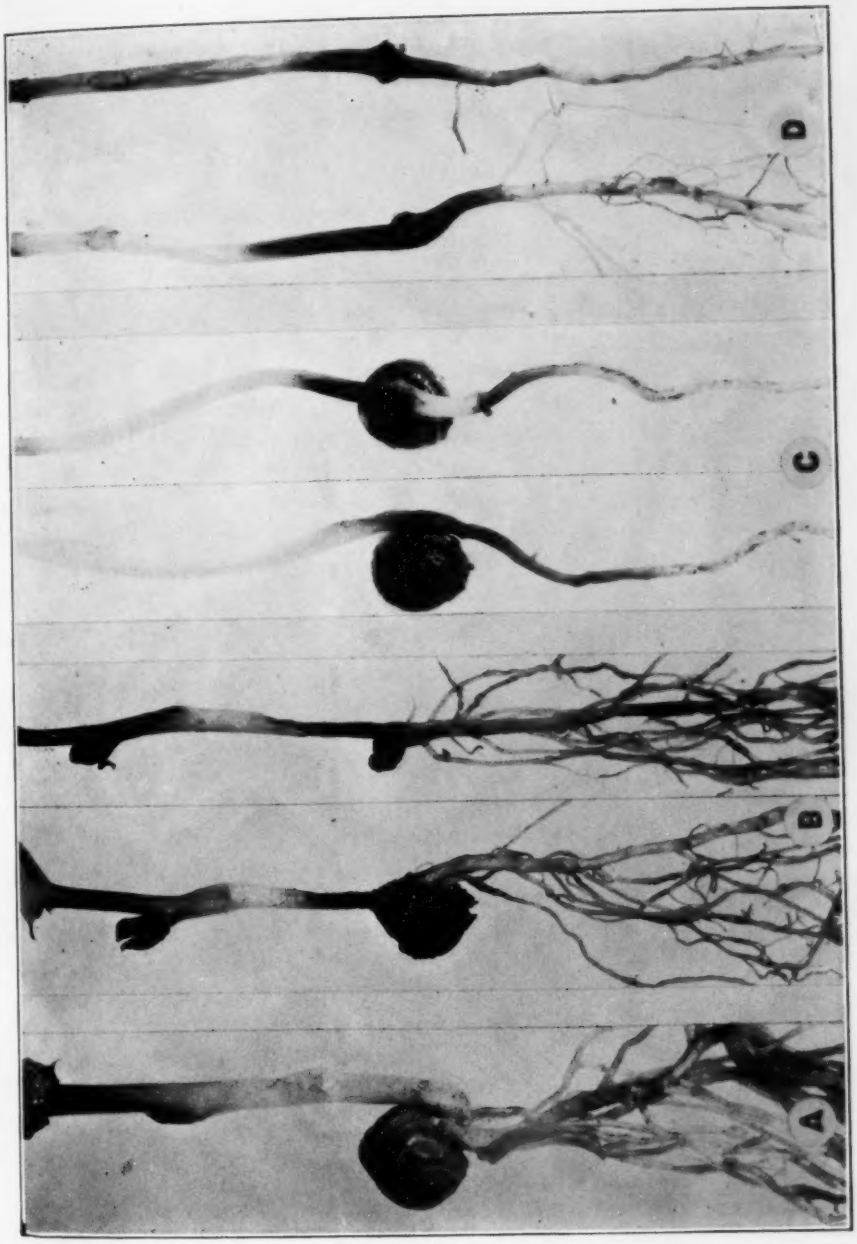
Photographs of pea plants showing lesions at the base of the epicotyl caused by *Rhizoctonia solani*, *Ascochyta* sp. and *Fusarium martii* var. *pisi*.

A. Healthy pea plant.

B. Pea plants attacked by *Rhizoctonia solani* in the field.

C. Pea plants attacked by *Ascochyta* sp.—grown in inoculated soil in the greenhouse.

D. Pea plants attacked by *Fusarium martii* var. *pisi*—grown in inoculated soil in the greenhouse.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LITTLE LEAF DISEASE OF PECANS[1]

J. B. DEMAREE

INTRODUCTION

A disease of the pecan, here provisionally named "little-leaf,"[2] which is widely different from the commonly known diseases of this tree, was first observed by the writer in Ocean Springs, Miss., in 1920. The following year, pecan trees affected with the same disease were found in Gainesville, Fla. In 1924 the disease was observed on trees growing in yards in Biloxi, Miss. In addition to the diseased trees seen by the writer, affected leaves have been received from Darien and Savannah, Ga. Reports of "little-leaf" have come from two sources in Savannah. Dr. O. F. Burger, University of Florida, reports that he saw a pecan tree affected with this disease in Miami, Fla.; and Dr. L. E. Miles, A. & M. College, Auburn, Ala., has informed the writer of the occurrence of "little-leaf" on a pecan tree at Troy, Ala. Matz[3] undoubtedly observed the same trouble in Gainesville, Fla., in 1917. In his bulletin he illustrated what appears to be a typical case of pecan "little-leaf."

DESCRIPTION

A normal pecan leaf is composed of from 11 to 15 leaflets and varies from 8 to 20 inches in length. The leaflets are falcate, oblong-lanceolate, serrate, and range from 3 to 6 inches in length. Leaves affected with the "little-leaf," as a rule, are composed of a reduced number of leaflets, occasionally as few as two (fig. 1) and averaging 8 or 9 to the leaf. The terminal leaflet is often absent on affected leaves, giving them an even number of leaflets. The shape of an abnormal leaflet varies from almost round to oblong, and lacks the sickleshaped and pointed apex, which is very characteristic of the normal leaflet. The apical portion is often dead, dry, and ragged. The serrations of diseased leaflets are also less pronounced than those of normal leaflets. In more accentuated cases, the leaflets are both shortened and narrowed, the width being reduced to about one-third inch. The leaf rachis is also reduced in length, and many leaves are only one to

[1] Paper read before Southern Section, American Phytopathological Society, meeting with the Association of Southern Agricultural Workers, Atlanta, Ga., Feb. 3, 1925.

[2] The disease is known locally in south Mississippi as "mouse-ear."

[3] Matz, J. Diseases and insect pests of pecans. Fla. Agr. Exp. Sta., Bul. 147: 135–163. 1918.

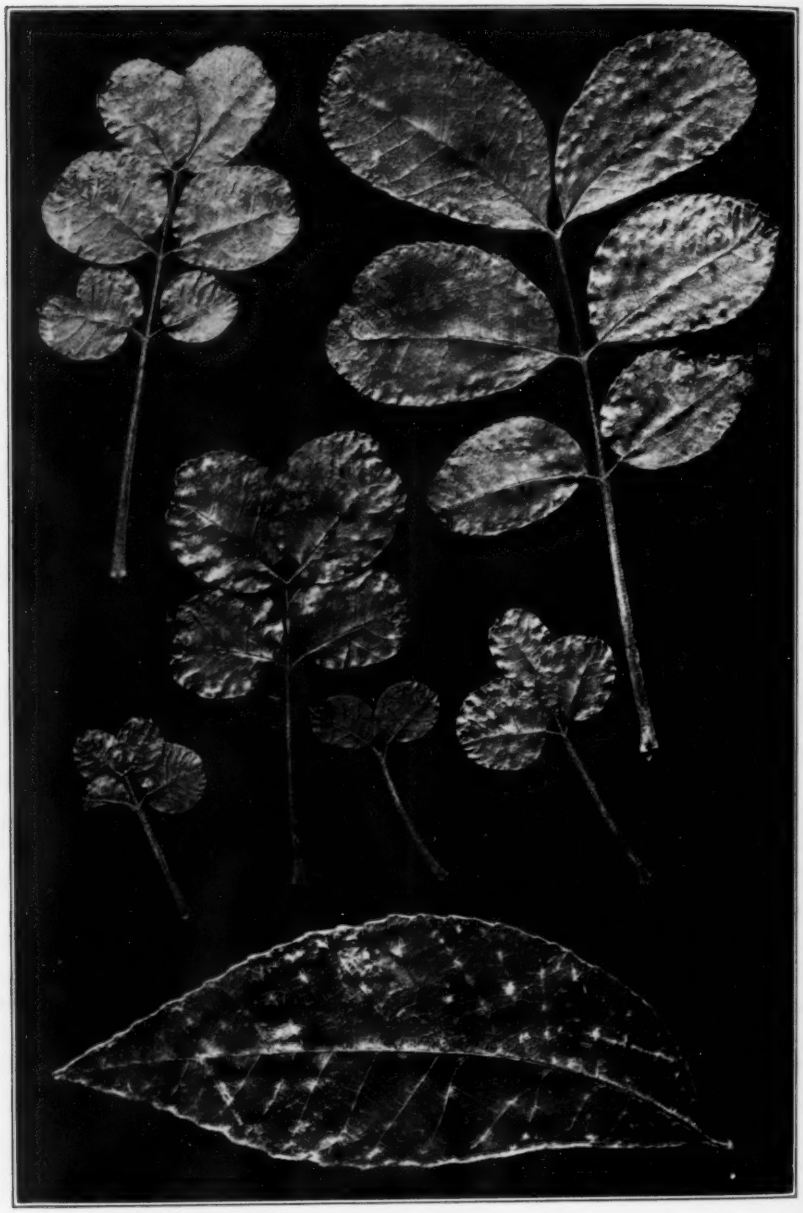

FIG. 1. Pecan leaves affected with the "little-leaf" disease. Note size and shape of leaflets in comparison with normal (lower) leaflet. Natural size.

two inches long, exclusive of the petiole. A slightly affected leaflet may be normal in size, the only visible symptom of the disease being the obtuse apex.

Badly affected twigs are extremely short and slender, with internodes correspondingly shortened (fig. 2). Buds on affected twigs are weak, undersized, and bluntly pointed. Those near the terminal end of the twig fail to develop. The general appearance of diseased buds and their frequent failure to resume growth the following spring indicate they are lacking in vitality.

Some trees observed were so severely affected that all leaves produced on the trees were composed of leaflets of the extremely rounded and diminutive type. The disease in other trees may be found only on certain branches and will recur each year on the same limbs, other limbs of the same trees producing normal leaves and twigs. Badly affected trees do not bear nuts, and their growth is greatly retarded. The writer has evidence that some trees recover after one or more years, while it is definitely known that the trouble has persisted in others for a number of years.[4]

DISTRIBUTION

It is remarkable that the disease is not known to occur under orchard conditions, but all cases so far observed have been in trees growing in gardens and lawns within city limits, and, with one exception (Troy, Ala.), confined to localities close to the Atlantic or Gulf Coasts. While no extensive study has been undertaken to locate the distribution of the "little leaf disease" of the pecan, in a general way it may be said to be present from Savannah, Ga., south to Miami, Fla., and west to Biloxi, Miss. It is believed that the disease will later be found in many other towns, especially those situated near the Atlantic and Gulf Coasts. A larger number of affected trees are known to be in Ocean Springs, Miss., and Gainesville, Fla., than at other points.

Hodgson[5] reports a "little leaf" disease of deciduous fruit trees as being prevalent in California. This western type of "little leaf" is characterized by the failure of the trees to leaf out at the normal time and, when they finally are formed, they are very small and narrow, and yellow in color. This condition may involve the entire tree or it may be confined to one or two limbs only. The appearance and effect varies somewhat on different kinds of fruit trees. In the peach, this disease is characterized by

[4] According to Mr. Theo. Bechtel, pecan grower and nurseryman, Ocean Springs, Miss., one tree at least has been known to have been affected continuously for the past fifteen years.

[5] Hodgson, Robert W. "Little leaf" of deciduous fruits. Mo. Bul. Calif. State Comm. of Hort. 7: 529–532. 1918.

the production of long, slender shoots on the new growth, terminated by a cluster or tuft of leaves. The small leaves along the branch usually are shed relatively early in the season. In the apple and pear, "little leaf" usually manifests itself more in the dwarfing of the leaves on the affected

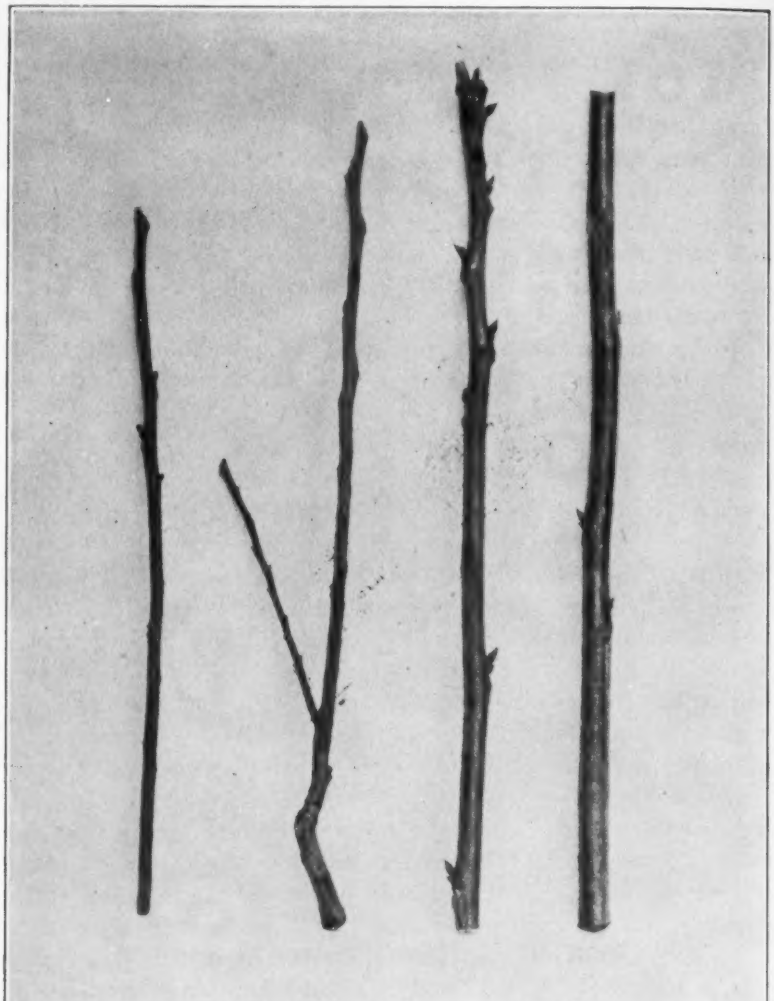

FIG. 2. Photograph of pecan twigs. Two on left affected with "little-leaf." Two normal twigs on right. ½ nat. size.

branches. In the walnut, the effect consists usually in greatly delayed leafing out, the resulting leaves being small and decidedly mottled in appearance.

The cause of "little leaf" of deciduous fruit trees in California is attributed to unseasonable climatic conditions, such as long, dry winters. There is a decided correlation, as reported by Hodgson, between the presence of the California "little leaf" and light sandy or arid soil.

PROBABLE CAUSE OF THE DISEASE

Very little is known of the factor or factors which induce the development of pecan "little-leaf." The temperature, rainfall, soil, and other environmental conditions are quite similar at all points where the disease is known or reported to be present. There has been no correlation observed between its prevalence and a deficiency of soil moisture, as is reported in the case of the California "little leaf" disease.

There is no indication that the disease is transmissible, except that in two towns, Ocean Springs, Miss., and Gainesville, Fla., where the disease has been known to be present for a number of years, an unusually large number of diseased trees have been found. Two or three diseased trees are sometimes found on the same lot, garden, or lawn, but, in such cases, some condition of the soil may be the predisposing factor. Healthy trees are known to have stood within a distance of 50 feet of a badly affected tree for a period of from 12 to 15 years without showing any indication of infection.

As the factor causing the diminutive leaves and slender twigs was thought by some growers to be in the branches, pruning the affected trees severely and burning the severed parts were tried in an effort to eradicate the disease. No favorable results were obtained: the new leaves growing from the stubs were diseased also.

Matz[6] attributes the abnormal leaves to low temperature injury to the buds after they begin to swell. The writer has not observed any correlation between this condition of pecan leaves and the weather, and does not believe that the trouble is caused by late frosts, as suggested by Matz. A leaf trouble caused by frost injury would necessarily only affect those leaves appearing during early spring. But when a tree is badly affected with "little leaf," even those leaves that are formed at the ends of growing twigs during mid-summer may have the reduced number of small round or oblong leaflets so characteristic of the disease. Then, too, frost would not likely cause this type of trouble in the same trees annually over a period of from twelve to fifteen years. The possibility of frost injuring the leaf buds on one or two limbs of a tree would be remote. Since the trouble has been found only in trees growing in the extremely fertile soils usually prevalent in old established lawns and vegetable gardens, it may be due to the action of some injurious soil substance there.

[6] Matz, J. *Ibid.*

EXPERIMENTAL WORK

The question arose as to whether the disease could be disseminated by using diseased scions or buds in propagation of nursery trees. During February, 1923, 24 scions taken from a badly diseased tree in Ocean Springs, Miss., were grafted on one-year-old nursery seedlings at Thomasville, Ga. Those twigs used did not have the vigorous, plump appearance of normal twigs. They had more the appearance of short, slender switches than vigorous, promising scions. The contrast is well illustrated in fig. 2. Although the grafting was done by an experienced nurseryman of many years' success, and special care was taken in placing and tying the scions on the stock, only 3 of the 24 lived. The growth resulting from these 3 living scions was normal in every respect.

In July, 1923, an attempt was made to transmit the disease by budding. Twelve buds taken from diseased twigs were inserted in the twigs of young healthy trees. All of the buds except two died, and these produced normal vigorous branches.

In April, 1924, 25 scions from a tree affected with "little leaf" were cleft-grafted in healthy five-year-old trees. Remembering the failure of slender switch-like scions in the year previous, more vigorous appearing twigs were used. Those were selected, however, that showed signs of disease as evidenced by the small blunt buds. As a result of this trial, 9 lived and produced healthy branches, while the remainder failed to grow. It is also worth while to record here that the contact of the scions or buds with the stock did not in any instance transmit the disease-producing element to the stock. While the attempts that have been made to transmit the disease to healthy trees cannot be considered as conclusive on account of the limited number of trials made, the evidence suggests that the disease is not likely to be transmitted by the ordinary nursery propagating methods. During February, 1924, a six-year-old pecan tree located at Ocean Springs, Miss., that had been badly affected with "little-leaf" for three years previous, was transferred by the writer's request to a point outside of the city and replanted in an Orangeburg type of soil in an environment approximating field conditions. The tree continued to live and showed slight symptoms of "little leaf" during the season of 1924. The tree was again kept under observation during the season of 1925, and not the slightest trace of the disease could be found.

SUMMARY

A disease affecting the leaves and twigs of the pecan tree has been under observation since 1920 and is here named "little leaf."

Affected leaves, as a rule, are composed of a reduced number of leaflets, averaging about eight to the leaf and sometimes reduced to two. Affected

leaflets are oblong or rounded, often with obtuse or notched apices, and frequently are greatly reduced in size. Affected twigs are slender, lacking in vitality, and the buds are small and blunt.

This condition of pecan trees has been found in Savannah and Darien, Ga., Gainesville and Miami, Fla., Troy, Ala., and Ocean Springs and Biloxi, Miss. The disease has been found only within city limits and never under orchard conditions.

Unsuccessful attempts were made to transmit the disease by budding and grafting. A badly affected tree transplanted to a location approximating field conditions entirely recovered.

The cause of the disease is not definitely known.

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RELATION OF OXYGEN TO SPORE GERMINATION IN SOME SPECIES OF THE PERONOSPORALES

B. N. UPPAL[1]

INTRODUCTION

Oxygen is considered one of the chief requisites in the process of germination. It is well known that the seed of the phanerogam will not germinate if the requisite supply of oxygen is not available, even though suitable conditions of moisture and temperature prevail. It is also generally agreed that, in fungi where spores germinate by the production of a germ tube, the relation of oxygen to germination is essentially the same as in a germinating seed. However, the question arises, in how far does there exist in the conidia of the species of *Phytophthora* a physiological similarity in regard to the processes of direct and indirect germination, in relation to the requirements of oxygen? In other words, are we to consider the formation of zoospores as a process physiologically similar to that of the production of germ tubes? Moreover, in how far is the relation of oxygen to indirect germination a characteristic of other forms of the Peronosporales?

Ward (5) considered that the liberation of zoospores was favored by the presence of free oxygen. Melhus (2) concluded that there was sufficient oxygen in the conidium of *Phytophthora infestans* to permit germination without the presence of free oxygen in water. Murphy (3) observed that the presence of yeasts and bacteria in culture arrested germination of the conidia of *Phytophthora infestans*. He seemed to think that the competing organisms used up oxygen in the water, thus retarding germination of the sporangia. He used various organisms to contaminate the cultures, and, judging from the similarity of results obtained, inclined to the view that the question of excretory products was not involved. The conflicting evidence as to the role of oxygen in indirect germination suggested that it might be profitable to attack this problem anew.

TRIALS IN WHICH THE AIR WAS REMOVED WITH A VACUUM PUMP

A series of experiments was planned to determine whether or not the conidia of *Phytophthora colocasiae*[2] germinate in the absence of oxygen in

[1] The writer takes great pleasure in expressing his gratitude to Professor I. E. Melhus, under whom this work was done, for the many valuable suggestions and helpful criticisms given during the progress of this work.

[2] My sincere thanks are due to Dr. William MacRae, Imperial Mycologist to the Government of India, and to Dr. N. J. Giddings, of the West Virginia Station, for supplying me with cultures of the species of *Phytophthora*.

the surrounding atmosphere. The first trials were made in an atmosphere from which the air had been exhausted by means of a vacuum pump. Microscopic glass culture slides, one by three inches, with two polished circular concavities, each measuring 18 mm. in diameter and approximately 1.75 mm. in depth, were used for growing cultures. Double distilled water was boiled to drive out any absorbed air, and cooled rapidly to the temperature of the room. The concavities on the slides were filled with the distilled, cooled water, to which the spores were then added. The culture slides were placed in a desiccator over 200 cc. of boiled water.

The desiccator was connected to a vacuum pump and exhausted, until the air within it had been so much attenuated that it could hardly support a six mm. mercury column. Exhaustion of the desiccator was continued for another five minutes. In a short time the water at the bottom of the desiccator, becoming agitated briskly, was changed rapidly into water vapor, producing a misty atmosphere within. This water vapor helped to wash out the last traces of air in the desiccator, in which the vapor pressure was 19.630 mm. of mercury at 22° C.

The desiccator was placed outside on a window sill, on a wet, cool (10–15° C.) night. Checks were made similarly in another desiccator, except that no air was removed. At the end of two hours none of the spores in the oxygen-free desiccator had germinated, while those in the checks had germinated profusely and zoospores were still being formed.

The following day the same experiment was repeated. The desiccators were kept in a compartment of an automatically controlled refrigerator, registering 11–12 ° C., and the cultures were examined after seven hours. Careful examination of the cultures in the oxygen-free desiccator revealed a few empty conidia but only one or two zoospores; probably the rest had disintegrated.

At the end of the experiment the cultures to which a few drops of water were added (since they had almost dried up) were placed on a table at room temperature (22° C.) for 30 minutes. They were examined after this period, and many of the spores germinated and produced zoospores. Evidently, then, the conidia were not injured and had retained their power of germination.

It was observed in the experiments just described that, after an exposure of one hour or so to conditions within the desiccators, the culture drops had almost dried up. Naturally the idea suggested itself that this drying of the culture drops may have had something to do with the inability of the conidia to germinate in an oxygen-free atmosphere. To obviate this difficulty, syracuse watch glasses, about two and one-half inches in diameter, were used in place of microscopic culture slides.

As in the previous experiments, double distilled water was boiled, rapidly cooled, poured into the syracuse watch glass to fill one-third of its volume and charged with the spores. The rest of the treatment was similar to that described in the previous experiments, except that two desiccators were used, one of which was opened for examination after seven hours and the other after nine hours.

When the cultures in the oxygen-free desiccators were examined zoospores were abundant, a few of which were still moving about rather sluggishly. After about seven minutes at room temperature (24° C.), most of the ungerminated conidia in the cultures had started forming zoospores.

The following day the same experiment was repeated, but when the vacuum pump had so much attenuated the air within the desiccators that there was barely enough pressure to support a five mm. mercury column, exhaustion of one desiccator was continued for 12 minutes and that of the other for 25 minutes, after which they were stored in a refrigerator at 11–12° C. for six hours. When examined after this period, almost 100 per cent of the conidia had germinated by zoospores. The experiment was repeated with similar results.

TRIALS IN WHICH OXYGEN WAS REMOVED FROM THE AIR IN THE DESICCATOR

Although there was no doubt that exhaustion of the air from the desiccators was as complete as could be obtained under the conditions of the experiment, it seemed advisable to remove the oxygen with an alkaline solution of pyrogallic acid.

In two trials two desiccators were set up with the spores of *Phytophthora colocasiae* sown in boiled, distilled water in syracuse watch glasses. For the elimination of oxygen from these desiccators, an alkaline solution of pyrogallol was used, which was prepared in the two following ways: first, a solution of one part of pyrogallol in three parts of water was mixed with an equal volume of a solution of one part of potassium hydroxide in three parts of water. This solution, when freshly prepared, absorbs 10 times its volume of oxygen, and has been designated as Berthelot's solution of pyrogallol (6). Second, a solution was made with one gram of pyrogallol and 100 cc. of 10 per cent potash-water for every 100 cc. of air space. This solution will be referred to in this paper as Mace's solution of pyrogallol (4).

The desiccator receiving Berthelot's solution of pyrogallol had enough reagent to absorb one and three-quarters times the volume of oxygen that was actually present in the air of the desiccator. The other desiccator which received Mace's solution of pyrogallol had enough of the solution to absorb one and one-half times the volume of oxygen present in the air of the desiccator. Both the desiccators were allowed to stand at room tempera-

ture (24° C.) for 30 minutes before they were removed to a refrigerator at 11–12° C., for the absorption of oxygen is very slow at temperatures below 15° C., and it is also believed that when oxygen is absorbed slowly a large amount of carbon monoxide is produced (4). In this connection the author has made a number of experiments and has found that when desiccators containing an alkaline solution of pyrogallic acid were removed immediately to a refrigerator at 11–12° C. and allowed to stand there for from two to six hours, the gas inside the desiccators, tested by Orsat Gas Analysis Apparatus, usually showed from zero to two per cent of the volume of oxygen absorbed as carbon monoxide (1).

The cultures from both the desiccators were examined after six hours, and approximately 100 per cent of the conidia had germinated to produce zoospores. Checks gave similar results.

In another series of experiments two desiccators were set up, both receiving Berthelot's solution of pyrogallol for the elimination of oxygen. The reagent used was enough to absorb twice the volume of oxygen actually present in each desiccator. This amount was used as a precaution against accident. The desiccators were allowed to stand at room temperature (23° C.) for 30 minutes. One of the desiccators received no other treatment and was removed to a refrigerator at 11–12° C.; the other was connected to a vacuum pump and exhausted, until there was brisk agitation in the alkaline solution of pyrogallol in the bottom of the desiccator. The desiccator was then removed to the refrigerator. Examination of the cultures after six hours showed zoospores as abundant as in the check.

Another set of experiments was planned on the basis of the following hypothesis. If oxygen is essential for growth (as is generally accepted), and if direct germination is a form of growth, conidia of *Phytophthora colocasiae* in an oxygen-free atmosphere and at 29° C., or at higher temperatures, should not germinate by tube.

Two desiccators were set up, both receiving Berthelot's solution of pyrogallol for the elimination of oxygen. The reagent used was enough to absorb twice the volume of oxygen actually present in each desiccator. After standing at room temperature (22° C.) for 20 minutes, one desiccator received no other treatment and was removed to a thermo-electric incubator, in which the temperature was maintained at 30–31° C. The other desiccator was connected to a vacuum pump, the air exhausted in the usual manner, and the desiccator removed to the thermo-electric incubator.

The desiccator from which the air was not exhausted but had received the alkaline solution of pyrogallol was incubated for 12 hours. Upon examination it was found that the conidia in these cultures had not germinated during this period. The other desiccator, from which the air was exhausted, was incubated for 22 hours. Even in this case none of the conidia germi-

nated. In all checks the conidia germinated profusely by means of germ tubes. Direct germination, therefore, seems to require oxygen in the air around the spores. The experiment was repeated three times, with the difference that all cultures were examined after 24 hours. None of the conidia germinated, although checks always germinated well.

EXPERIMENTS WITH SOME OTHER SPECIES OF PHYTOPHTHORA

After having found that indirect germination in *Phytophthora colocasiae* was not dependent upon free oxygen in water, an attempt was made to ascertain whether the same relationship holds in the other species of *Phytophthora*. Three additional species, *Phytophthora infestans*, *P. palmivora*, and *P. parasitica*, therefore were used. *Phytophthora infestans* and *P. palmivora* were grown on oatmeal agar, the former also being grown on cut potato tubers. *Phytophthora parasitica* was cultivated on cornmeal, which was prepared by adding to the meal sufficient water to keep the medium moist after sterilization.

As in the experiments above described, Berthelot's solution of pyrogallol was used to remove oxygen from the desiccators, which were then allowed to stand at room temperature for 20 minutes before they were connected to an electric vacuum pump to exhaust the remaining air. Exhaustion of the desiccators was continued for 10 to 15 minutes after the air had been so much attenuated that it could hardly support a six mm. mercury column. The cultures were incubated at 11–12° C. for 18 hours. Examination after this period showed that zoospores had formed in all cultures in an oxygen-free atmosphere. All checks germinated well; the percentage of germination in cultures of *Phytophthora infestans* was about 50 per cent. These experiments were repeated three times at three different periods with essentially similar results.

In another series of experiments with the three species of *Phytophthora*, Berthelot's solution of pyrogallol was used to remove oxygen from the desiccators, which, however, were not exhausted by a vacuum pump. The results on germination were the same as in the previous experiment.

EXPERIMENTS WITH SOME SPECIES OF PLASMOPARA, PERONOSPORA, SCLEROSPORA AND ALBUGO

The peculiar response of indirect germination of the conidia of the species of *Phytophthora* to lack of free oxygen around the cultures was so interesting that it was deemed desirable to extend these observations to spore germination in the other species of the Peronosporales. Spore germination in *Albugo*, *Plasmopara*, *Peronospora* and *Sclerospora*, was studied.

The method of removing oxygen from the desiccators, in which cultures were placed, was the same as that employed in the above experiments. The

conidia of *Sclerospora graminicola, Plasmopara viticola* and *Albugo candida* failed to germinate in cultures surrounded by an oxygen-free atmosphere. The checks always germinated well, except that in *Albugo candida* germination of the conidia varied greatly, and was always less than 50 per cent. The conidia of *Peronospora trifoliorum,* which germinate by the production of germ tubes, also failed to germinate in the oxygen-free desiccators.

DISCUSSION

The experimental data above presented seem to indicate the following relationships of oxygen to spore germination in the Peronosporales: 1) except in *Phytophthora,* direct and indirect germination of the spores of the species of the Peronosporales is dependent upon free oxygen, other conditions for germination being optimum; 2) in *Phytophthora* indirect germination is more or less a result of the effect of temperature, and is independent of the relation of oxygen, at least in so far as the atmospheric oxygen is concerned; and 3) direct and indirect germination are two different phenomena. The former is a type of growth, while the latter is nothing but the breaking up of the intersporangial protoplasm aroused to activity by the invigorating influence of temperature, into independent protoplasmic units, each unit being endowed with the same power of direct germination as is a conidium under certain conditions.

That indirect germination in *Phytophthora* is independent of the relation of free oxygen is contrary to the observations of Ward (5), Murphy (3), and others, who claimed for oxygen a stimulating influence upon this type of germination. However, the contention of Melhus (2) that "free oxygen in the water is unnecessary for germination, and . . . the conidium contains within itself sufficient oxygen for germination," seems worthy of examination. Granting that there is sufficient oxygen within the sporangium to insure its germination when placed in water and under the right conditions of temperature, it seems difficult to explain why direct germination, which seems to be dependent upon free oxygen, as is shown by the present author's experiments, failed to occur when the cultures were placed in an oxygen-free atmosphere at 30–31° C. Of course it may be argued that, in this genus, the requirements of oxygen for the two types of germination are so different that, while the oxygen within the sporangium may stimulate one type of germination, it may be incapable of influencing the other without the aid of free oxygen in water. It is, however, for future research to show whether such is really the case.

In arriving at the third conclusion that, in *Phytophthora,* direct and indirect germination are two different phenomena, it may be questioned whether such a broad generalization seems warrantable from the experi-

mental data at hand. However, if we consider the material changes which occur in the process of spore germination in general, it will be realized that the process with which we are dealing in indirect germination of the conidia of the species of *Phytophthora* is hardly comparable to the one which occurs, for example, in the rusts. Considerable amounts of organic food materials are stored in all spores. During germination the germ tube utilizes the stored food material and continues its development as long as the reserve food in the spore is available. If, however, the culture medium does not contain any food material, the germ tube is hindered in its development and ultimately starved to death. Now if we may look upon the formation of zoospores as nothing but the rearrangement of cytoplasm followed by cleavage, it will seem reasonable to assume that the products of cleavage, viz., zoospores, should retain the food material necessary to sustain the growth of germ tubes. But if we may regard the liberation of zoospores as a process comparable to the production of germ tubes, it will follow that the process of indirect germination has deprived the conidium of its stored energy (in the form of reserve food material), and the zoospores are incapable of further development, just as a germ tube is, unless supplied with nourishment from the medium. But the zoospores, as we know, act as independent protoplasmic units endowed with the same power of producing a germ tube and sustaining its growth for a short time that is possessed by a conidium. Evidently, then, a zoospore is a physiologic equivalent of a conidium insofar as its ability to produce and sustain a germ tube is concerned. The process of the liberation of zoospores in *Phytophthora,* therefore, is not germination in the same sense as that process in a germinating rust spore; it is a process which prepares the conidium to multiply itself vegetatively, a fact very significant as an aid in the dispersal of the spores. A zoospore is, in fact, a sporangiospore, and the process which gives rise to it is the formation of sporangiospores.

It is very important to note that the process of indirect germination in *Phytophthora* is not the same as that in the other genera of the Peronosporales. In what ways a change in the physical influences of the surrounding medium will affect the complex material system within a sporangium of a species of *Phytophthora* and not that of the other genera of the Peronosporales is not yet easy to comprehend. It may be that the cleavage of the protoplasm in the sporangia of *Phytophthora* is more complete than in the other genera of the Peronosporales, so that the attempt on the part of a sporangium of this genus to throw out a part, or the whole, of its cytoplasm takes much less energy than is required in the other genera. It should also be mentioned in passing that it would be of great significance to examine some species of algae in order to determine whether the formation of zoospores in sporangia is influenced by the presence or absence of oxygen around the cultures.

SUMMARY

Conidia of *Phytophthora colocasiae* under proper temperature conditions were able to form zoospores in cultures from which the air had been exhausted by means of a vacuum pump. They also formed zoospores in cultures from which the oxygen was withdrawn by alkaline solutions of pyrogallic acid.

Indirect germination also occurred in the case of conidia of *Phytophthora infestans, P. palmivora,* and *P. parasitica* in the absence of oxygen.

Direct germination did not occur with the conidia of any of the species of *Phytophthora* used, when oxygen was excluded from the cultures.

The conidia of *Albugo candida, Plasmopara viticola,* and *Sclerospora graminicola,* which germinate indirectly, as well as the conidia of *Peronospora parasitica* and *Peronospora trifoliorum* which germinate directly, required the presence of oxygen for germination.

Direct and indirect germination are two different phenomena, the former being a type of growth, the latter a mere breaking up of the protoplasm into independent units.

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LITERATURE CITED

(1) CALVERT, F., S. CLOEZ and BOUSSINGAULT. Ueber die Bildung von Kohlenoxyd bei der Einwirkung von Sauerstoff auf pyrogallussaure Kali. Liebig's Ann. Chem. **130**: 248. 1864.

(2) MELHUS, I. E. Germination and infection with the fungus of the late blight of potato (*Phytophthora infestans*). Wis. Agr. Exp. Sta. Res. Bul. 37: 1–64. 1915.

(3) MURPHY, P. A. The bionomics of the conidia of *Phytophthora infestans* (Mont.) de Bary. Sci. Proc. Roy. Dublin Soc. n. s. **16**: 442–466. 1922.

(4) SMITH, E. F. Bacteria in relation to plant diseases. Carnegie Instit. Publ. 27: 59. 1905.

(5) WARD, H. M. Illustrations of the structure and life history of *Phytophthora infestans,* the fungus causing the potato disease. Quart. Jour. Micros. Sci. (London) n. s. **27**: 413–425. 1887.

(6) WHITE, A. H. Technical gas and fuel analysis. New York. p. 319. 1920.

EXOBASIDIUM CAUCASICUM WORONICH. IN TRANSCAUCASIA AND KAMCHATKA

N. N. Woronichin

W. Tranzschel, in a personal conversation with me, expressed the supposition that the fungus, *Exobasidium caucasicum*, found on the leaves of *Rhododendron caucasicum* Pall. at the top of of the Zchra-Zkaro (Transcaucasia), and described by me (12), is possibly identical with the specimens of *Exobasidium* collected by the Academician, V. Komarov, in Kamchatka on the leaves of *Rhododendron chrysanthum* Pall.[1] and determined by W. Tranzschel as an *Exobasidium pentasporium* Shir. (11, p. 570). Therefore I again looked through the material of *Exobasidium caucasicum*, from Kamchatka, collected by the Academician V. Komarov, and the specimens of *Exobasidium pentasporium* Shir., collected on *Rhododendron rhombicum* Mig. in Nicco, determined by the author (from the herbarium of the Section of Mycology and Phytopathology, A. A. Jaczewski) and by others on the *Rhododendron sp.* from the collection of Samurai in the herbarium of the Botanical Museum of the Academy of Sciences, Tokyo.

I regret that it was impossible to study the structure of the basidia and spores (the latter, according to the description, are similar in dimension and shape to those of *Exobasidium caucasicum*) of the last two specimens because of the poor conservation of the hymenium of the parasite; but comparing the diagnostic description and drawings of Shirai (7, p. 53, Pl. IV, fig. 18–19), with the external aspect of the infection, it was possible to determine the undoubted difference between my *Exobasidium* and the fungus of Shirai, which may be seen from table 1.

Concerning the specimens from Kamchatka, they are identical with mine as to the exterior aspect of the infection and also as to the microscopical characters. I succeeded in observing but little difference as to the dimensions of basidia and basidiospores. The basidia of the specimens from Kamchatka measured 42–52 × 7–10 μ against 39.6–42.9 × 6.6–8.8 μ of the specimens from Transcaucasia, but the basidiospores of the specimens from Kamchatka measured 14–17.5 × 5–5.5 μ, so that they scarcely differed from those of the Caucasian forms, the dimensions of which are 14–16.5 × 5 μ. The number of spores on the basidia of the fungus from

[1] Specimen labeled "*Exobasidium pentasporium* Shir. on *Rhododendron chrysanthum* Pall. Kamtchatka, from Petroparlovsk to Kamtschakta Mt., near the vil. Ganal: the subalpine zone. June 18, 1909. Lg. V. Komarov."

Kamchatka was 4, while their shape is identical with that of *Exobasidium caucasicum*. It was often observed that the spores are septate and that they bud, producing fusiform conidia with little oil drops at the ends; the conidia are somewhat larger than those on the Caucasian specimens, reaching 7–10 × 1.75–3 μ, mostly 7 × 1.5 μ compared with 5 × 1.5 μ in the diagnosis of *Exobasidium caucasicum*.

TABLE 1.—*Comparison of Exobasidium caucasicum and Exobasidium pentasporium*

Structure	*Exobasidium caucasicum* Woronich.	*Exobasidium pentasporium* Shir
Hymenium	Constantly dispersed on the lower surface of the young leaves.	In most cases in patches, more seldom dispersed on the lower surface of the leaves of different age, sometimes leaving their margin free.
Basidiospores	4, seldom 2–3	4–6, commonly 5.
Branches	Does not form "witches' brooms"	Forms "witches' brooms"
Host plants	*Eurhododendron*	*Azalea* and *Tsusia*.

The discovery of my fungus on *Rhododendron chrysanthum* in Kamchatka, seems to me, clears up the question of the origin of the Caucasian *Rhododendron*.

Prof. N. Kusenezow presents the history of the genus *Rhododendron* in Caucasia as follows: "Concerning the other Ericaceae of the Flora Caucasica, they probably have developed in the Caucasus in the post-tertiary time and can be considered as . . . the further development of the tertiary types of Caucasia, e.g., *Rhododendron caucasicum, R. ungernii, R. smirnovii* and others, which changed after the tertiary time and became adapted to the new climatic environment in Caucasia after the ice period." (3, p. xi.) Such an ancient tertiary type of *Rhododendron* in Caucasus is *Rhododendron ponticum* L. according to Kusnezow, "an ancient type, near to the original tertiary form of the Caucasian *Rhododendron*, or, perhaps, identical with one of them. From such an ancient type, on the boundaries of Colchids and Armenia, probably came the endemical Caucasian Rhododendrons, *R. ungernii* Trautv. and *R. smirnovii* Trautv. From the same type, *R. caucasicum* Pall. arose in the mountains of Caucasus, and *R. chrysanthum* Pall. in the mountains of Siberia. The ancient type itself, *i.e.*, *R. ponticum*, has remained in Colchid." (3, p. 13.)

The fact of the parasitism of *Exobasidium caucasicum* on *Rhododendron chrysanthum* on the one hand and its occurrence on one species of the Caucasian Rhododendrons, namely *R. caucasicum*, on the other hand does not

quite agree, I believe, with the aforementioned description of the genesis of the group of Rhododendrons in Caucasia.

In fact, excluding the possibility of transferring the parasite by atmospheric currents in the present, or even at a more ancient time, and recognizing our representative of the genus *Exobasidium* as an ancient type of fungus, it would be natural to find it parasitizing the primordial species of *Rhododendron* or the closely related species, *R. ponticum* L.

Such cases of parasitism of *Exobasidium caucasicum* on *R. ponticum* have not been observed up to this time, although this fungus is not rare in Transcaucasia and is often found, as evidenced by N. Troitzky's and my own observations.

I think that the facts of parasitism and the extreme resemblance between *R. caucasicum* and *R. chrysanthum* as shown by Maximowicz (4, p. 21); the similar ecology of the two species; and a somewhat taxonomic separatism of *R. caucasicum* from the other two species of this genus, known in Caucasus,[2] admit the endemism (or more exactly hemiendemism) of *R. caucasicum* as not equivalent to the endemism of the other two endemes of the genus *Rhododendron* in Caucasus. It seems to me possible to admit the migration into Caucasus of *R. chrysanthum* as such during one of the ice periods, perhaps together with the other fourteen emigrants from the high mountains of Asia as enumerated by Kusnezow (3, p. xxi). In its new habitat this species could be the progenitor of the contemporary *R. caucasicum* in the same manner as the arctic species of *Gentiana* have formed their separate races in Caucasus (3, p. xxvii).

The example, described by me, of the parasitism of *Exobasidium caucasicum* on two closely related, although geographically separated, species of *Rhododendron* recalls an analogous case, stated by Raciborski, which gave to this author the opportunity of expressing the following considerations concerning the particular occurrence of *Azalea pontica* L. (otherwise called *Rhododendron flavum* Don.) in Eurasia. This bush is known to grow in the Caucasus, in Asia Minor, in the North of Volhynia near Ovruteh, and in the year 1909 it was found also in the forest of Sandomir. In the latter country Raciborski has found in abundance on the leaves of *Azalea* the parent fungus, *Exobasidium discoideum* Ell., for the first time described in America on the related species of *Azalea viscosa* L. An almost identical form of this fungus, *Exobasidium discoideum* var. *horvathianum* Thom., has been found on the leaves of *Azalea pontica* L. in Caucasus.

[2] *Rhododendron ungernii* and *R. smirnovii* can be put between *R. ponticum* and *R. caucasicum*. Prof. Kusnezow, p. 22 of the publication cited, says: "The position of *Rhododendron ungernii* and *R. smirnovii* between *R. ponticum* and *R. caucasicum* as well as their geographical distribution give some reason for supposing that these two new forms are a hybrid of secondary generations."

Azalea pontica L., and *Azalea viscosa* L., correspond taxonomically with the representatives of the group "Azaleae genuinae," which grow in greatest abundance in North America and only a few of which occur in the East of Asia. The relationship of the North American Azaleae and *Azalea pontica*, according to Raciborski, is demonstrated by the parasitism of the above mentioned *Exobasidium*, which undoubtedly developed on the primordial native Azaleae and has extended from there together with the different species of Azaleae in North American and Eurasiatic countries. Raciborski assumes that there could have been during the Myocene such a center of development of Azaleae in the extreme North, from whence the species extended to the South, in the direction of the meridians. It is interesting to remark that on the leaves of Azaleae in the forest of Sandomir, Raciborski found also the other form of *Exobasidium* whose independence from *Exobasidium discoideum* Ell. he doubted (5, p. 391). This fungus was insufficiently described later by Raciborski in "Mycotheca polonica" under the name *Exobasidium dubium* Rac. (nomen nudum) (6, p. 1172).[3] At the same time this fungus was found by some other explorers in Anatolia and in Transcaucasia; a little later it was found in several countries of western Transcaucasia. In the Caucasian lists, the fungus was designated as *Exobasidium sp.*, and in the work of Bubák as *Exobasidium vaccinii* (Zûl.) Woronin. Forma. *Rhododendri flavi.* Bub. (1, p. 101). In the year 1913 the organism was thoroughly studied by me and named *Exobasidium magnusii* (13, p. 15). And so this species forms, as to its occurrence, a full parallelism with *Exobasidium discoideum* Ell., but apparently has not been observed in America up to this time.

Although these parallel but geographically separated forms of *Exobasidium* are fixed by me under three specific names, nevertheless it is possible that in each of these three species a more detailed study will establish morphological differences in accordance with the geographical distribution.

The measurements mentioned by me in the beginning of this treatise show in fact some unimportant differences in the dimensions of basidia and conidia, on the species of Caucasian and Kamchatkan *Exobasidium caucasicum*. The statement of Raciborski (5, p. 388) for the *Exobasidium dubium* differs a little as to the dimensions of basidia from the Caucasian *Exobasidium magnusii*, but, according to the information of Thomas (10, p. 305) the Caucasian specimen of *Exobasidium discoideum* differs from the American species by the still smaller dimensions of basidiospores which compelled the author to designate the Caucasian form under the name, var. *horvathianum*. I am sorry I could not find in the literature exact informa-

[3] Specimen labeled *Exobasidium dubium* Rac. (*Exobasidium* sp. Raciborski *Azalea pontica* 1909, p. 398, fig. 2.) Na lisciach *Azalea pontica* w Woli Zarzyckiej w puszczy Sandomierskiej. 20/6. 1909 Zb. M. Raciborski.

tion as to the dimensions of basidia and basidiospores of *Exobasidium discoideum* (5, p. 385, 8, p. 665, 9, p. 538). Burt (11, p. 645) gives the dimensions of the basidiospores only.

In general the question of the existence of morphological differences between the geographically isolated pairs of *Exobasidium* needs special study, but it is not improbable that in this case we have to deal with three pairs of elementary species, which differ in their distribution and in some unimportant morphological characters.

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LENINGRAD, RUSSIA.

LITERATURE CITED

1. BUBÁK. Fungi in Handel-Mazzetti, Botanische Reise in das pontische Randgebirge. Ann. Naturhist. Hofmus. Wien, **23**: 101–108. 1909.
2. BURT, E. A. The Thelephoraceae of North America IV, Ann. Missouri Bot. Gard. **2**: 1915.
3. KUSNEZOW, N., N. BUSCH, and S. FOMIN. Flora Caucasica critica **4**: Pt. 1. 1–128. 1901. I–LXII. 1908.
4. MAXIMOWICZ, C. J. Rhododendrae Asiae Orientalis. Mem. Acad. Sci. St. Petersburg Ser. VII. **16**: No. 9. 1870.
5. RACIBORSKI, M. *Azalea pontica* in Sandomirer Wald und ihre Parasiten. Bul. Internat. Acad. Sci. Cracovie, Cl. math. Natur. 1909. Sem. 2. 1910.
6. ———. Mycotheca polonica Kosmos *XXXIV*: 1909.
7. SHIRAI, M. Description of some new Japanese species of *Exobasidium*. Bot. Mag. Tokyo. **10**: No. 113, Pt. II. 1896.
8. SACCARDO, P. Sylloge fungorum **6**: 1888.
9. SOMMIER ET LEVIER. Enumeratio plantarum annus 1890 in Caucaso lectarum. Acta Horti Petropolitani **16**: 1900.
10. THOMAS, F. Ueber einige Exobasidien und Exoasceen. Forstl. Naturw. Zeitschr. **6**: 305–314. 1897.
11. TRANZSCHEL, W. Die Pilze und Myxomyceten Kamtschatka's. Expedition a Kamtschatka, organ. par Th. P. Riaboushinsky Soc. Bot. **2**: 1914.
12. WORONICHIN, N. Notes mycologiques II. Moniteur Jard. Bot. Tiflis **51**: 1920.
13. ———. Mycoflorae Caucasicae novitates. Moniteur Jard. Bot. Tiflis **28**: 1913.

TRANSMISSION OF POTATO SPINDLE-TUBER BY CUTTING KNIVES AND SEED PIECE CONTACT[1]

R. W. Goss

INTRODUCTION

The spindle-tuber disease repeatedly has been found to increase more rapidly in certain infected seed stocks in Nebraska than could be accounted for by the presence of known insect carriers in the field. While this increase in the disease possibly might be due to the presence of undetermined insect carriers, it was thought that seed piece contact and the cutting knife might serve as a means of transmission. The only reported attempts to secure infection in this way, with which the author is familiar, were made by Schultz and Folsom,[2] who report that they were unable to infect healthy tubers by means of the cutting knife in a limited number of tests. They also found that juice from infected tubers was not as virulent as juice from the green parts of the plant when leaf mutilation inoculations were made. The results would indicate that the infectious principle may not be in an actively virulent condition in the tuber, but we know, on the other hand, that infected tubers usually produce infected plants and that tuber grafts usually give a high percentage of infection. Tuber inoculations made by the author with plugs of infected tissue inserted into healthy seed pieces always have given a high percentage of infection. It appears then that the infectious principle present in the tuber is in a virulent state and capable of causing the infection of healthy seed pieces.

No attempt was made in this work to distinguish between spindle-tuber and unmottled curly dwarf as the symptoms of these two diseases have not been clearly differentiated on the Bliss Triumph variety. It is possible that unmottled curly dwarf as described by Schultz and Folsom was present in these tests.

A preliminary test was made in the greenhouse by cutting a healthy tuber into 4 seed pieces and inoculating 3 of them by rubbing the freshly cut surfaces with spindle-tuber seed pieces two or three times. The 4 seed

[1] Published with the approval of the Director as Paper No. 15, Journal Series, of the Nebraska Agricultural Experiment Station, Lincoln, Neb.

[2] Schultz, E. S. and D. Folsom. Infection and dissemination experiments with degeneration diseases of potatoes. Observations in 1923. Jour. Agr. Res. 30: 493–528. 1925.

pieces were planted in separate pots. All 3 inoculated seed pieces produced spindle-tuber plants, and the new tubers showed the characteristic symptoms of the disease (Plate XXI, A). The control seed piece produced a healthy plant with normal tubers.

EXPERIMENTAL METHODS

Further tests were made in the field in 1925, using both contact and knife inoculations. Healthy tubers were halved; one half was cut into 2 quarters with a knife disinfected in lysol, and these quarters were used for controls. An infected tuber was then cut in half with another knife, and this knife rubbed once across the freshly cut surface of the healthy half tuber and then used to cut it into 2 quarters. The procedure with the contact inoculations was similar, except that a sterile knife was used on each tuber and 2 of the quarters were rubbed once or twice with the freshly cut seed pieces of infected tubers. The inoculated seed piece, the healthy control from the same tuber, and the diseased control used as the source of inoculum were planted opposite each other in 3 rows, the healthy controls in the middle row. All the seed pieces were planted immediately after inoculation. The soil was moist, and conditions were ideal for sprouting. The plot was sprayed with Bordeaux mixture, containing both lead arsenate and nicotine sulphate, as often as necessary to keep out insects. Emergence records were taken and notes made every ten days. The progeny of each hill was harvested separately so that both yield and tuber symptoms of each inoculated plant could be compared with its healthy and diseased control plant. Fifty plants for each method of inoculation were grown in each row. The Bliss Triumph variety of potatoes was used in all the tests.

RESULTS

No differences were noted between the inoculated and healthy plants as to time of emergence; and the diseased controls sprouted almost as rapidly, probably owing to the ideal soil conditions at planting time.

The symptoms of the disease appeared on the spindle-tuber controls as soon as the plants were large enough to enable the symptoms to be distinguished. The inoculated plants which became infected had clearly observable symptoms of the disease a month after emergence.

Ten of the healthy control plants in each plot had symptoms of the disease both in the tops and the tubers; these plants, with the corresponding inoculated plants which were all infected, were discarded and not considered in the data from which table 1 was compiled.

As seen in table 1, infection was obtained by both methods of inoculation (Plate XXI, B and C), but a larger percentage of infection resulted

from the contact inoculation. All the plants listed as infected had the disease symptoms appearing on the tops, and the degree of severity of the symptoms on the tops corresponded with those on the tubers. The infected plants had a smaller total yield than the healthy controls, the average of both tests being only 52 per cent of the yield of the controls. There was also a smaller percentage of marketable potatoes from infected plants, both tests averaging only 42 per cent of the controls.

In addition to the infected plants listed in table 1, 19 plants, or 47.5 per cent of the inoculated plants in the knife inoculation test, and 18 plants, or 45 per cent of the contact inoculations, were questionable spindle-tuber. These plants showed some symptoms of the disease in the field, and the tubers were not entirely normal. The total yield per plant was only 83 per cent, and the yield of No. 1 tubers only 77 per cent of that of the healthy controls. The symptoms of the disease were not distinct enough to group them with the infected plants, although undoubtedly most of them would produce distinct spindle-tuber plants the following year.

There were thus only 8 plants, or 20 per cent of the plants inoculated with the cutting knife, which remained healthy on the same basis as the healthy controls, while only 3, or 7.5 per cent of the contact inoculations, remained healthy.

As stated above, 10 of the 50 controls showed typical spindle-tuber and were not considered in the above data. The corresponding inoculated plants also showed the disease, but in a more severe form; the yield was only half that of the infected controls, and the tuber symptoms were much more severe. It is possible that this may have been due to the additional inoculation of these infected seed pieces.

The contact inoculations not only gave a higher percentage of infection than the knife inoculations, but also resulted in more severe symptoms of the disease. The yield per hill was much less, and the symptoms on the tubers were more severe, as shown in table 1. This probably was not due to the method of inoculation, however, as the spindle-tuber controls also show the same differences. The half of the rows in which the contact inoculation set was grown received a little more irrigation water, and the soil moisture content was undoubtedly higher throughout the season. This is evidenced by the comparative yields of the healthy controls, which were 100 to 200 grams per hill higher in the well watered part of the plot. It is known that a high soil moisture content tends to increase the severity of the tuber symptoms of spindle-tuber, and the above differences probably can be accounted for on this basis.

DISCUSSION

The chief sources of error in an inoculation experiment of this type are reduced to a minimum in the experiments here reported. The possibility

TABLE 1.—*The results of inoculating healthy seed pieces with spindle-tuber by means of the cutting knife and by seed piece contact*

Treatment	No. of plants	Per cent of plants infected	Aver. total weight in grams of tubers per plant	Aver. wt. in grams of No. 1 tubers per plant	Total number of tubers	Tuber Symptoms[a]						
						H	?	1	2	3	4	5
						Per cent of total number of tubers						
Spindle-tuber controls	50	100.0	168	34	333	0	0	0	2.7	37.8	40.5	18.9
Healthy controls	40	0	1072	952	451	100	0	0	0	0	0	0
Inoculated by means of cutting knife[b]	40	32.5	814	694	122	28.7	4.9	26.2	22.1	11.4	6.5	0
Spindle-tuber controls	50	100.0	148	68	212	0	0	0	0	26.9	44.8	28.3
Healthy controls	40	0	1237	1085	497	100	0	0	0	0	0	0
Inoculated by seed piece contact[b]	40	47.5	391	175	199	3.0	6.0	3.0	24.6	31.1	19.6	12.6

[a] H = healthy; ? = questionable; 1 = very slight; 5 = very severe.

[b] All the data on weight, number, and symptoms of inoculated plants are based on the number of infected plants.

of the original seed tubers being infected is hardly plausible when the presence of disease in the inoculated row is between 30 and 50 per cent, with the controls from the same tubers remaining healthy. The possibility of current season infection would hardly account for the results, inasmuch as the control row was planted between the inoculated and diseased rows and all doubtful controls were discarded along with the plants from the corresponding inoculated seed pieces.

We must therefore conclude that spindle-tuber can be transmitted by cutting knives and by contact of freshly cut seed pieces. It is possible, also, that a picker planter could serve as a means of transmission. No evidence is at hand, however, to prove whether or not this actually occurs in practice, but the author can see no reason why the disease could not be spread in this way.

If this means of transmission is proved to be a factor in the spread of the disease, it will call for additional control methods. The sterilization of cutting knives is impractical, but the very rigid sorting out of all spindle-tuber seed before cutting would decrease this danger. It is possible, also, that healthy seed pieces which have come in contact with infected ones or with cutting knives used to cut infected tubers could be treated after cutting with a disinfectant and the control of the disease be increased in this way.

SUMMARY

Evidence is presented here which shows that the spindle-tuber disease of potatoes may be transmitted by cutting knives and by contact of freshly cut seed pieces. The results of the experiments may have an important bearing on methods of control.

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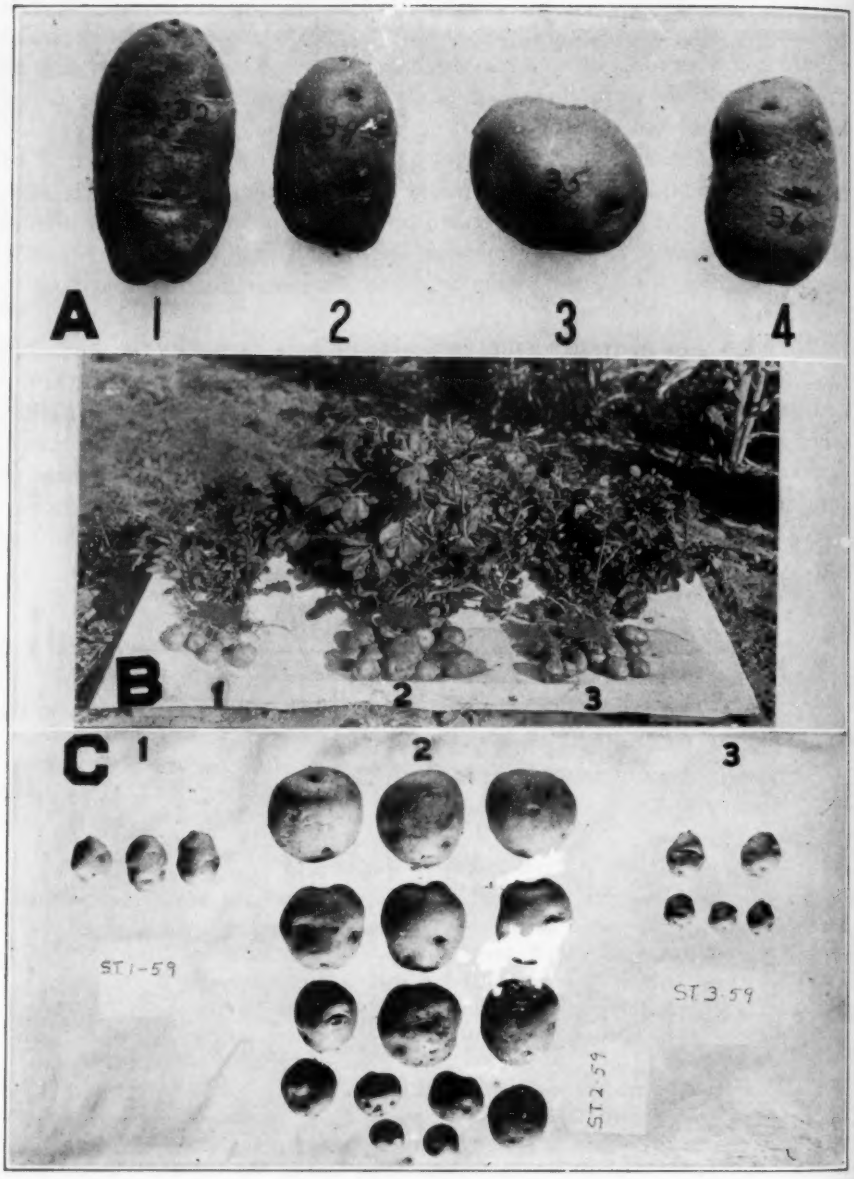
EXPLANATION OF PLATE XXI

A.—Results of inoculations in the greenhouse made by rubbing together the freshly cut surfaces of spindle-tuber and healthy seed pieces. Tubers 1 and 2, progeny of 2 inoculated seed pieces. Tuber 3, progeny of healthy control from the same tuber as 1 and 2. Tuber 4, progeny of spindle-tuber seed used as the source of inoculum.

B.—Plants and tubers from cutting-knife inoculation test in the field. Plant 1, spindle-tuber control from seed used as source of inoculum; Plant 2, healthy control from same tuber as Plant 3, which was grown from a seed piece inoculated by cutting with a knife used to cut the seed piece for Plant 1.

C.—Progeny of seed-piece-contact inoculation test in the field. 1, progeny of spindle-tuber seed piece used as source of inoculum; 2, progeny of healthy control seed piece; 3, progeny of inoculated seed piece. The healthy control and the inoculated plant were from seed pieces from the same tuber.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THE FUSARIOSIS OF CEREAL CROPS IN EUROPEAN RUSSIA IN 1923

M. DOUNIN

FUSARIOSIS OF SMALL GRAINS

The summer of 1923 was extraordinarily rainy and it had many cloudy days. This probably accounts for the widespread prevalence of fungi of the genus *Fusarium* Lk. on the cereal crops of Russia. Numerous specimens obtained from different parts of the country give reason to believe that this fungus was disseminated in 1923 over a very considerable territory, the western boundary of which almost coincides with the western boundary of Russia. The western boundary is about the 52d meridian (from Greenwich); whereas the northern and southern boundaries extend from about the 62d to the 54th parallels, respectively.

In different localities of this region the percentage of infected grains varied considerably. It diminished southward and eastward. Most of the infected specimens were obtained from the region between Lake Ladoga and Lake Onega, and also eastward from the latter, covering the territory between the 32d and the 35th meridians east longitude and the 62d and 60th parallels north latitude.

In connection with the extraordinary prevalence of *Fusarium roseum* Lk. on rye, it was observed that the bread became poisonous (Inebriant bread). People who ate it suffered from weakness, vertigo, headache, nausea, and vomition. Table 1 shows the percentages of infected kernels in the specimens obtained from the region designated above. Besides the unusually high percentage of infected kernels, table 1 also points out that, in most of the infected grains, the fungus was found in a dead condition.

The above specimens were examined under the microscope immediately after the grain was harvested, and the infected kernels were then kept in a moist chamber for a period of from seven and one-half to eight months. If the cereals had been kept in the usual way so considerable a number of Fusaria (average 65 per cent) would not have died.

By studying the "biography" of each specimen we found out that the only way in which the Fusaria could be killed without damaging the grain was by drying the grain before threshing. The peasants of the above-mentioned region dry their grain in drying-sheds built especially for this purpose. These sheds are heated by stoves without chimneys. The sheaves

are put in the drying sheds and kept there for 12–18 hours at a temperature of from 46 to 54° C. The effect of the heat on the Fusaria is probably enhanced by such products of combustion as HCOH, SO_2, CO, etc., because of the absence of chimneys and because the door is kept tightly closed until the fuel is wholly burnt up.

Experiments were undertaken which showed that, after drying the grain in the described manner, a large percentage of the Fusaria were dead. It is possible that, under certain conditions, the drying-sheds may become a means of control against the fusariosis of cereals.

TABLE 1.—*The percentage of Fusarium-infection in grain crops in European Russia in 1923*

Specimen number	Infected crop	Percentage of infected kernels			
		Total	Percentage of kernels with viable Fusaria		Percentage of kernels with dead Fusaria
			Before chemical disinfection	After chemical disinfection	
1	Oats	17	1	0	16
2	Rye	92	30	0	62
4	Rye	56	12	1	44
5	Barley	90	7	3	83
6	Rye	91	1	2	90
7	Oats	27	16	12	11
8	Rye	100	1	0	99
9	Rye	90	19	8	71
10	Oats	89	30	16	59
11	Oats	89	10	5	79
12	Rye	77	6	2	71
13	Rye	92	10	5	82
14	Rye	80	35	17	45
Average		73			65

An investigation of the degree of infection was undertaken. It has been shown that many, if not most of the kernels, are infected with Fusaria after harvesting when the sheaves are kept in shocks set up in the field.

The mycelium is found mostly in the superficial strata of the kernels, as oftentimes has been noted by many authors. A careful study of the character of mycelial distribution in the superficial strata of the kernels has shown that, in the specimens examined, the mycelium is not uniformly distributed. Most frequently it was found in the ends of the kernels, as is illustrated in table 2.

If we assume that the brush end of the kernel is not a good infection court during the ripening period and, in general, previous to harvesting,

TABLE 2.—*The percentage of Fusarium-infection in different parts of grain kernels*

Specimen number	Percentage of infection				Differences between		
	Entire kernel	Brush end of kernels	Middle portion of kernels	Germ end of kernels	3 and 4	3 and 5	5 and 4
			Rye				
2	92	92	66	90	26	2	24
4	56	56	44	44	12	12	0
6	91	91	82	91	9	0	9
8	100	100	89	66	11	34	−23
9	90	90	90	80	0	10	−10
12	77	37	44	77	−7	−40	33
13	92	92	92	92	0	0	0
14	80	80	70	70	10	10	0
Average	85	78.5	72	76	6.5	2.5	4
			Oats				
1	17	17	0	17	17	0	15
3	36	36	14	29	22	7	15
7	27	27	18	9	9	18	− 9
10	89	89	89	89	0	0	0
11	89	89	89	77	0	12	−12
Average	52	52	42	44	10	8	2
			Barley				
5	90	90	80	90	10	0	10

then we may consider the figures in column six as some indication of the way in which the Fusarium fungus penetrates the kernels after gathering.

INEBRIANT LINSEED-OIL

We also have obtained information concerning "inebriant linseed-oil" from the Gomel region. Specimens of flax seed from which the poisonous oil was obtained were found infected with a special form of *Fusarium*. A pure culture of this form was obtained, and, when compared with *Fusarium lini* Boll., it offered essential differences in the way of development of the

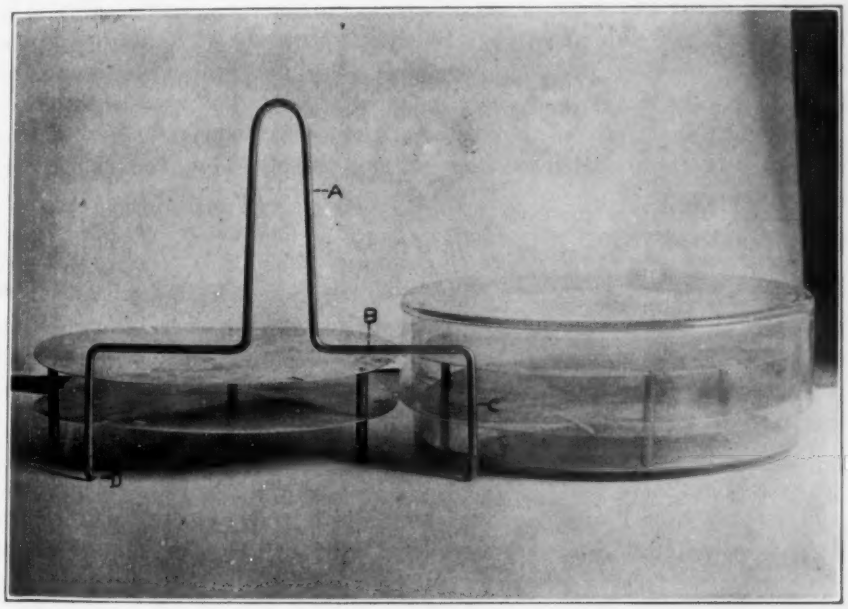
mycelium, in its color on nutritive media, and in the way of conidial formation.

As no other poisonous matter was found in these specimens of flax seed, and as the symptoms of poisoning by "inebriant linseed oil" are quite similar to those caused by "inebriant bread" (due to *Fusarium roseum*), this new form of *Fusarium* most probably is the cause of the inebriant characters of linseed oil which the population of certain regions of Ukraina use as a food product.

MOSCOW, RUSSIA.

PHYTOPATHOLOGICAL NOTES

Device for Increasing the Capacity of Moist Chambers.—The writer has found the following device useful in increasing the capacity of moist chambers where such are to contain culture slides or objects of somewhat similar bulk such as small Petri dishes. Essentially it consists of two zinc disks, one of which is supported from the bottom of the dish by three brass pins, and the second similarly upon the first one. The unit is removed from the moist-chamber or replaced by means of a lifter. While a similar equipment serving the purpose may be made more simply, there is added a description of this apparatus as used by the writer. It may be readily made from materials in the laboratory mechanician's shop, and is of permanent utility.



The dimensions given are those of the device fitting a glass moist chamber with a lid of 23 cm. inside diameter and with a tray of 21.5 cm. inside diameter. Two circular disks of zinc one half mm. in thickness are cut to a diameter of 21.2 cm. Slots are cut in each disk at opposite edges to a depth of 12 mm. and a width of 6.5 mm. It is important that these slots have the same relative position on each disk. Three holes, each 3 mm. in diameter, are drilled at points 6 mm. from the edge of the disk and 17 cm. apart. (Lines connecting these holes form an equilateral triangle.) It

is also important that these holes have the same relative position on each disk. Supporting and connecting pins for the zinc disks are made from brass rods 6 mm. in diameter. Three are cut to a length of 2.6 cm. and one end of each shouldered to a length of 5.5 mm., that part of the pin being threaded to fit in the small holes drilled in the disk, and to screw into the ends of the next set of pins. The latter three are 2.2 cm. in length, are drilled at one end to accommodate the pin set below them, and have a short shoulder at the other end to protrude through the small holes in the zinc disk resting upon them. These parts then are assembled as indicated in the accompanying figure.

The device is most conveniently removed from the dish for examination of materials by means of a lifter, made of flexible brass wire, or rod, 4 mm. in diameter, of the shape indicated in the illustration, and the free ends of which are obliquely faced off. Section A of the lifter has a length of about 12 cm.; B, 8 cm.; C, 6 cm.; while the oblique facing at D has a length of 3 mm. In using the lifter, its broad end is inserted in the slots of the zinc disks and the upper ends are compressed, which causes the lifter to engage the slots at their inner edge, when the arrangement may be brought out *in toto*. The top disk is then easily taken off, and the contents of both are available for examination.—N. M. GRIER, Dartmouth College, Hanover, N. H.

Occurrence of Club Root on Chinese Cabbage.—In a recent paper, W. H Davis[1] reported Chinese cabbage (*Brassica pe-tsai* Bailey) as a new host for the club root organism (*Plasmodiophora brassicae* Wor.). The disease was reported on this plant in 1912 by Cunningham[2] who considered it to be one of the seven most susceptible of the twenty-eight species studied.—F. L. WELLMAN, University of Wisconsin.

Results of Dutch Investigations.—In the forthcoming 32d volume of the "Tijdschrift over Plantenziekten" new results of Dutch investigations on diseases of potatoes, beans, cabbage, and other field and garden crops and some notes on forest pathology, will be published. The more important papers will contain summaries in English (sometimes in French or German).

Editors: Prof. Dr. Ritzema Bos, forester De Koning, Ir. v. Poeteren of the Plant Disease Survey, and Prof. Dr. Quanjer of the Institute for Phytopathology. Business manager: Dr. H. J. Calkoen, Dieren, Holland. Subscription price is $2.30 per year.—H. M. QUANJER.

[1] Davis, W. H. Club-root of Chinese cabbage. Mycologia 17: 160–162. 1925.

[2] Cunningham, G. C. The comparative susceptibility of cruciferous plants to *Plasmodiophora brassicae*. Phytopath. 2: 138–142. 1912.